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Eumelanin-Pheomelanin

EVIDENCE FOR INDEPENDENT MODULATION OF PROLIFERATION AND MELANIZATION IN S91 MELANOMA CELLS BY PROSTAGLANDINS (PGs). Z. Abdel Malek, V. Swope, N. Amornsiripanitch, J. Nordlund, Department of Dermatology, University of Cincinnati College of Medicine, Cincinnati, Ohio.

The Cloudman S91 melanoma cell line, CCL 53.1, responded to PGE_1 and PGE_2 in a dose-dependent manner by an increase in tyrosinase activity and by inhibition of proliferation. These cells responded to PGA_1 and PGD_2 by decreased tyrosinase activity and proliferation, but were not affected by $PGF_{2}\alpha$. PGE_1 (10µg/ml) enhanced tyrosinase activity 12 hours after treatment; an effect that was blocked by actinomycin D, cycloheximide, and phenylthiocarbamide (PTU). PGE_1 (10µ /ml) inhibited proliferation within 4 hours after treatment. However, this effect was not abrogated by concomitant treatment with actinomycin D, cycloheximide, or PTU. Dibutyryl cyclic AMP (db cAMP) and isobutylmethylxanthine (IBMX) greatly augmented the activation of tyrosinase by PGE_1, but did not enhance the inhibitory action of PGE_1 on cell growth. We conclude that only some PGs affect S91 melanoma cells each in a specific manner. PGE_1-induced stimulation of tyrosinase activity required de novo transcription and translation. Blockage or enhancement of the PGE_1 effect on tyrosinase does not alter the PGE_1-induced retardation of proliferation. These observations strongly imply that melanogenesis and proliferation of CCL 53.1 melanoma cells are regulated by independent mechanisms. In addition, we suggest that PGA_1 and/or PGD_2 may function as inhibitors of melanization.

PHOSPHORYLATED MIXED ISOMERS OF L-DOPA INCREASE MELANIN IN SKINS OF SKH-2 HAIRLESS MICE. P. Agin and J. Pawelek, Schering-Plough Corporation, Memphis, TN and Yale University School of Medicine, New Haven, CT

A new class of compounds, "dopa-phosphates", with phosphate ester linkages at the 3- and/or 4-positions of the phenylalanine ring increase pigment in hairless mouse epidermis. Groups of mice were painted with varying concentrations of dopa-phosphate (DP) daily for 5 weeks. Control groups were painted with 0.1M tris-glycerol buffer containing phenylalanine or L-dopa. Half of the groups received suberythemal irradiation 3 times weekly for 4 weeks from filtered FS20 lamps, resulting in minimal epidermal pigmentation. DP painted groups without irradiation achieved modest pigmentation which was concentration dependent. Application of buffer alone did not produce additional pigmentation without irradiation. Optimal concentration of DP was 0.01%, above and below which the amount of pigmentation induced was decreased. With added irradiation, the 0.01% DP painted group achieved pigmentation above that of the irradiated controls. At higher and lower concentrations the enhancement effect was less pronounced. DP appears to stimulate the production of melanin and affect the development and distribution of melanocytes, both with and without irradiation. This may be effected by a process similar to that seen in melanoma cells, which incorporate dopa-phosphates into melanin, presumably following enzymatic hydrolysis by phosphatases with resultant production of L-dopa and inorganic phosphate.

THE GORDON-KOSSWIG MELANOMA SYSTEM IN 1986
Annerose Anders and Fritz Anders
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Xiphophorus, which was introduced by Gordon and Kosswig in 1928 as a system for melanoma research is abundantly equipped with cellular oncogenes: c-erb, c-src, c-myc, c-yes, c-abl, c-fgr, c-fes, c-myb, c-ras, c-sis, c-fos. The c-oncs, although highly conserved during evolution, differ to a certain degree within the genus. Restriction enzyme patterns of several c-oncs of a given species are male- and female-specific indicating that the X and Y chromosomes contain different copies of the respective c-onc. Many of the c-oncs are active from the outset of cleavage all over the life of an animal with very high activities during organogenesis, and different activities in different organs. It appears that many of the c-oncs operate differently at different times and for different purposes during the course of development. Development of melanoma which occurs spontaneously following crossings or following carcinogen treatment of hybrids is in parallel with oncogene amplification, with elevation of oncogene activity, and with elevation of RNA-dependent DNA polymerase activity. Out of 8 differently organized copies of c-src only 2 appear as the "critical copies" for melanoma formation. -The c-oncs have probably evolved in parallel with the large taxa of the metazoa.

A MELANOGENIC FACTOR PRODUCED BY MELANOCYTES OF THE FIBROMELANOTIC (FB) PHENOTYPE IN CHICKENS. **Billye W. Auclair, and J. Robert Smyth, Jr., University of Massachusetts, Amherst, MA 01003.**

Fibromelanosis (FB) in chickens is characterized by extensive pigment deposition in mesodermal tissue, and results from an interaction involving two genes. One, id^+, is responsible for the deposition of pigment in shank dermis, this phenotype being designated PS (pigmented shanks). The other, Fb, in the presence of id^+ extends pigment deposition in mesodermal tissue throughout the body. The Id allele inhibits melanization of these tissues, and represents a control phenotype (C).

In this experiment, melanocyte cultures were obtained from neural tube explants of FB, PS, and C 2½-day-old embryos. When compared for differences in the number of melanin-producing cells, FB and PS cultures were found to produce approximately ten-fold more pigmented cells/culture at 72 hrs. of incubation than did C cultures ($P<.05$). When media conditioned by FB melanocytes for 48 hrs. was added to FB, PS, or C neural tube cultures, a significant increase in the number of pigmented melanocytes was observed when compared with melanocyte cultures treated with conditioned media from either PS or C cultures ($P<.05$). This indicates that the FB melanogenic factor differs either qualitatively or quantitatively from substances produced by PS and MI cultures. When media conditioned by FB melanocytes was added to fibroblast cultures, there was no stimulation of fibroblast growth, suggesting that the FB melanogenic factor is not a general cell growth enhancer.

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IDENTIFYING CUTANEOUS AND UVEAL MELANOCYTES IN CULTURES FROM AVIAN NEURAL TUBES. Raymond E. Boissy. Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Neural-crest derived melanocytes populate two anatomical sites: the epidermis of regenerating feathers and the uvea of eyes. These two populations can be distinguished by morphologic and functional criteria. Feather and uveal melanocytes synthesize structurally different melanosomes. Feather melanosomes are oval, approximately 0.3 μm x 0.8 μm, whereas uveal melanosomes are more spherical and twice as large. Feather melanocytes, after recruitment into the follicle, synthesize melanosomes continuously and transfer them to keratinocytes. Ocular melanocytes, after populating the uvea and becoming congested with melanosomes, remain melanogenically dormant and do not transfer granules.

Cultures of melanocytes established from embryonic neural tubes contain two morphologically different populations of melanocytes which resemble the two types in situ. Both types migrate from the entire length of the tube and continue to do so during several embryonic stages. Various mutant lines of chicken express alterations in pigmentation, and the cultured melanocytes also express these genetic defects. Most striking are white chickens with black eyes, carrying the c allele at the C locus. These birds have tyrosinase negative feather melanocytes and pigmented ocular melanocytes. Cultures from this line consist of a mixture of amelanotic and pigmented melanocytes. These findings suggest that cutaneous and uveal melanocyte populations can be cultured from neural tubes and distingished morphologically.

THE AVIAN MELANOCYTE. Roger R. Bowers, Department of Biology, California State University, Los Angeles, CA 90032 USA

Feather melanocytes and choroid melanophores originate in the neural crest whereas retina and iris pigment epithelium and pecten melanophores are derived from the optic cup. The mechanism of melanogenesis, elucidated primarily by studies of six pigment loci, consists of Golgi-derived coated vesicles containing tyrosine-tyrosinase complexes joining SER-formed rod shaped (eumelanin) or spherical (pheomelanin) protein fibrillar premelanosomes. Chicken tyrosinase, a probable glycoprotein found primarily in the insoluble fraction, has multiple forms and has dopa oxidase and tyrosine hydoxylase activity. Senescence and pigment enzyme pathways have been ultrastructurally and cytochemically studied in feather melanocytes. Pigment transfer to feather keratinocytes and associated lysosomal activity have been investigated. Recently methods for the establishment of tissue culture melanocytes from regenerating feathers and neural tubes have been reported. Heterokaryon formation in melanocytes has yielded information on the genetic control of avian pigmentation. Recently, chickens have been used as a model to study genetic hypomelanosis, including vitiligo. Supported by NIH MBRS 2S06RR08101-13.

DEVELOPMENTAL ASPECTS OF 5,6-DIHYDROXYINDOLE OXIDASE ACTIVITY IN AMPHIBIA. D.Botti,A.Arcadi*,A.Bonfigli,A.M.Cimini,A.Manilla,O.Zarivi, M.Miranda.Depts of Cell Biology &Physiology and of Chemistry Chemical Engineering & Materials*,University of L'Aquila,L'Aquila,Italy.

It was assumed,until recently,that tyrosinase (EC 1.14.18.1)catalyzes the first two reactions of melanin pathway.Increasing evidence indicates that tyrosinases from various sources also catalyze 5,6-DHI oxidation.

During Amphibian development tyrosinase is expressed after neural induction,when a new synthesis of enzyme occurs,and its kinetic and electrophoretic properties have been studied.

5,6-DHI oxidase activity has been studied in pre and postneurular stages of development in Bufo bufo :1)Km is of the same order of Km for L-DOPA and does not change during development;2)the activity is not PTU sensitive;3) the specific activity increases during development;4)two electrophoretic bands are always present.Since 5,6-DHI and L-DOPA oxidase activities comigrate,these results are strongly indicative of the existence of a different catalytic site for 5,6-DHI in Bufo tyrosinase.

ON THE SWITCH FROM PARTICULATE TO FIBRILLAR MELANOSOMES: MELANOGENESIS IN FISH. Roger R. Bowers, Department of Biology, California State University, Los Angeles, CA 90032 USA

In the wild type channel catfish Ictalurus punctatus, melanosomes are formed in the retina pigmented epithelium (RPE) and choroid melanophores (CM) by the deposition of melanin on fibrillar protein backbones, typical of higher invertebrate melanogenesis. A mutant, termed albinistic, has been reported in these catfish which has retarded melanogenesis in the RPE and CM and large particulate melanosomes are formed. Fibrillar premelanosomes are extremely rare. These particulate premelanosomes are formed by the fusion of electron dense melanin-containing vesicles arising from the Golgi cisternee or SER. Since the Stage II fibrillar premelanosomes are extremely rare in this mutant, it is concluded that these structures are melanized first and, when none of these fibrillar premelanosomes is present, then the melanin-containing vesicles fuse to form particulate melanosomes. Thus the switch from particulate to fibrillar type of melanosomes may be simply the evolutionary appearance of fibrillar Stage II premelanosomes. Literature reviews of melanogenesis in late melanizing goldfish, salamander oocytes, black moor goldfish, killifish hybrid melanomas, xiphophorin fish melanomas, guppy melanophores, squid ink glands and xanthic goldfish, support this conclusion. Fibrillar premalanosomes are rare or absent in these systems and particulate melanosomes are formed primarily. CSLA Inst. Grant A4177911.

EXPRESSION OF THE BARRING GENE IN FOWL: GENETIC HYPOMELANOSIS. R. R. Bowers, J. Harmon, J. Novoa, R. Schreck, and R. Rocha. Department of Biology, California State University, Los Angeles, CA 90032.

The sex linked barring gene, B, causes dilution of eumelanin pigmentation in the feathers, beaks, eye choroid and shanks of the Fowl. The decreased choroid pigmentation is due to either failure of melanoblast migration or failure of these cells to survive. In the Barred Plymouth Rock (BPR) chicken, this gene causes white bands to be deposited on an otherwise black feather background. Cytochemical and ultrastructural studies show that the BPR black band contains dendritic melanocytes active in melanogenesis. Melanocytes from between the distal black and proximal white band show ultrastructural changes indicative of degenerative cells with lower tyrosinase levels and higher acid phosphatase levels than those found in black bands. In the BPR white band, only a few degenerating melanocytes are found. Tissue culture and grafting techniques strongly suggest that the barring gene causes the feather melanocytes to be more sensitive than wild type melanocytes to a cytotoxic agent found in the black band which is always the first band to form in the BPR feather development. The dosage effect of this increased sensitivity is demonstrated two ways: 1) the B/B male fowl has 33% wider white bands than $B/+^{B}$; 2) the width of the subsequent white band is always proportional to the width of the preceding black band. The BPR Fowl may serve as a model of genetic hypomelanosis caused by a cytotoxic agent. (Supported by NIH MBRS 2S06RR08101-13).

MELANIN PIGMENTATION IN THE STRIA VASCULARIS John W. Conlee, Thomas N. Parks, and Donnell Creel, V.A. Medical Center, and University of Utah School of Medicine, Salt Lake City, UT, USA.

Evidence that reduced levels of cochlear melanin pigmentation is associated with increased auditory fatigue and a greater susceptibility to noise-induced hearing loss suggests a possible functional role for melanin pigment in the ear. We have studied the distribution of both melanin and lipofuscin pigments in the stria vascularis (SV) of several mammalian species. The density of strial melanin varies considerably among species and is negatively correlated with auditory threshold in some species. Although probably age-dependent, the least amount of melanin was found in the SV of the cat and the most in the guinea pig. In both light and electron microscopic studies we observed melanin pigment in the SV of all pigmented animals. Unlike lipofuscin, the melanin pigment did not fluoresce when examined with ultraviolet illumination. At the electron microscopic level, cells in the SV of pigmented animals displayed melanosomes with varying amounts of melanin.

The presence of strial pigmentation in all pigmented animals studied supports the possibility that melanin has a general role in mammalian cochlear function that is abolished by the albino and other hypopigmenting mutations.

COMPARATIVE STUDY OF MELANOSOMAL MEMBRANES FROM EQUINE EYES AND MELANOMA

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Membranes of transformed malignant cells show biochemical composition and physico-chemical characteristics different from normal ones, and some of these differences have been correlated with the growth rate of the tumors. Membranes of sub-cellular organelles should be also different in tumor cells and, in order to investigate this hypothesis, we have studied melanosomes from equine eyes and melanoma that show morphological as well as methabolic differences.
We have analyzed the composition in proteins, phospholipids, cholesterol and fatty acids of both intact melanosomes and isolated melanosomal membranes. Differences in dynamic and static parameters as rigidity and molecular order of the membranes, investigated by fluorescence depolarization and time-resolved fluorescence decay (using DPH as a probe) were determined and correlated with the biochemical data.

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LIPOFUSCIN (LF) AND MELANIN IN RETINAL PIGMENT EPITHELIUM (RPE) IN FELINE EYES.
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Macular degeneration, one of the most common causes of vision loss in adults, is usually accompanied by LF accumulation in the RPE. In human eyes the incidence of macular degeneration, and the amount of LF in the RPE are both inversely related to the amount of melanin in the RPE and/or choroid. To test the hypothesis that melanin protects the retina from light damage leading to LF, we compared RPE LF, and RPE and choroidal melanin in calico cats. Melanin content of the RPE varied continuously from OD = 0 over the tapetum, to OD = 0.8 Choroidal melanin exhibited discrete foci with increased (OD = .6) and decreased (OD = 0.3) pigment.

Specific observations which were made in both human and cat eyes included: (1) the LF and melanin contents of the RPE were inversely correlated; (2) RPE associated with darker choroidal pigment had less LF than cells over lighter choroid. (3) there was an age related dramatic increase in the LF content of RPE cells (especially over the tapetum in the cat) The increase in LF in the tapetal RPE can be attributed to the lack of RPE melanin, since the relationship between LF and RPE melanin is not altered. Thus the relationships observed between LF and melanin in different human eyes with variant light histories have been substantiated in single eyes of cats.

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MODIFICATIONS OF MELANOGENESIS AND THE STRUCTURE OF MELANINS BY THIOURACIL

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Thiouracil (TU) and related thioamides are widely used as antithyroid drugs. It was shown that they accumulate in melanotic tissues and radiolabelled TU was proposed to improve the diagnostic and therapeutic possibilities for malignant melanomas.

In this work the early steps of melanogenesis were studied in vitro with mushroom tyrosinase in the presence of melanin precursors and TU. Spectrophotometric and HPLC analyses of suitable substrates and products of catalysed reactions were used for determination of kinetic parameters of tyrosinase. The effect of TU on the modifications of the structure of melanins obtained by enzymatic oxidation and by autooxidation of DOPA and 5,6-dihydroxyindole (DHI) in the presence of TU was examined by the use of pyrolysis-gas chromatography-mass spectrometry, IR and ESR spectroscopy. The obtained results confirmed that incorporation of TU to melanins involves coupling of quinones derived from DOPA and DHI to TU, but simultaneously TU acts as a noncompetitive inhibitor of tyrosinase and inhibits to the same extent both the conversion of DOPA and DHI to quinones with a half-maximal concentration for inhibition of 0.03 mM.

CHARACTERIZATION OF THE THYROID PIGMENT INDUCED BY MINOCYCLINE THERAPY

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Minocycline is a tetracycline antibiotic used largely for the treatment of acne vulgaris. Thyroid pigmentation occurs as a rare and apparently benign side-effect of such therapy. Work in vitro has shown that this pigment probably is a polymer derived from the oxidation of minocycline. Like melanin, it is black, precipitates in acid, binds metal ions, and contains a stable pool of free radicals that can be studied with electron spin resonance (ESR) spectroscopy. However, comparison of the behavior of its ESR signal under various test conditions with the criteria used for identifying melanin shows this pigment to be unique. Similar to melanin, its signal intensity increases during illumination with visible light and in the presence of diamagnetic metal ions. Also characteristic of melanin, its signal intensity appears to decrease in the presence of paramagnetic metal ions. In contrast to melanin, however, no signal increase is found at high pH. From these and other data, it is concluded that the free radicals in pigment derived from minocycline are not semiquinones, as occur in melanin, but possibly are phenoxy radicals. Data are reported for both human thyroid tissue pigmented by minocycline and a synthetic model pigment from the in vitro autooxidation of minocycline.

PHOSPHORYLATION OF A MEMBRANE PROTEIN IN CLOUDMAN S91 MOUSE MELANOMA CELLS IS CONTROLLED BY α-MSH

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We have investigated a possible role of protein phosphorylation in the melanogenic action of α-MSH on Cloudman S91 mouse melanoma cells. Incubation of the cells with ^{32}P-phosphate resulted in the incorporation of the label into a large number of phosphoproteins. In the presence of α-MSH a significant increase of ^{32}P incorporation was observed into two phosphoproteins with apparent molecular weights of 43 kD and 34 kD, respectively. This increase was concentration dependent, reversible and could be induced by melanotropic peptides only. Subcellular fractionation of labeled melanoma cells and analysis of the proteins by PAGE revealed that the 34 kD protein is a membrane component whereas the 43 kD protein is of mitochondrial/melanosomal origin. Phosphorylation of the 34 kD protein is rapid which points to a participation in the receptor/adenylate cyclase-mediated signal transduction. This view is supported by structure-activity studies with a number of α-MSH analogs: the potency of the different peptides for inducing protein phosphorylation paralleled their activity in the tyrosinase assay. In addition, peptides which showed potentiating effects in the tyrosinase assay and in pigment cell assays were also potentiating α-MSH in its action on the phosphorylation of the 34 kD protein.

EFFECT OF ONTOGENY AND ENVIRONMENT ON COLOR PATTERN VARIATION IN THE ARIZONA TIGER SALAMANDER (AMBYSTOMA TIGRINUM NEBULOSUM HALLOWELL)

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Metamorphosed Ambystoma tigrinum Green show significant geographic variation in color pattern. Seven subspecies are recognized primarily on the basis of color pattern within distinct geographic ranges. Color pattern has inherent weakness as a taxonomic character in some species. The great variation among individuals, and individuals' ability to change color make it difficult to summarize the expected color pattern in some taxa. Dorsal spots on metamorphosed A.t. nebulosum Hallowell, for example, can be black, brown, olive, or yellow. Ground color, color of skin around spots, may be similarly colored so a range of color patterns occurs from black on yellow to yellow on black.

We assessed effect of microhabitat and ontogeny on color variation within and between 3 populations of A.t. nebulosum in Arizona. Our lab and field observations show that salamander color correlates with substrate color and water transparency. Larvae are dark on black substrate or in clear water. Larvae are light on white substrate or in turbid water. Larvae that are dark prior to metamorphosis transform into dark individuals and visa versa. Metamorphosed salamanders also change color, but much slower and to a lesser degree than larvae. As salamanders age they lighten in color and their ability to change color is reduced.

c-src ORGANIZATION AND AMPLIFICATION IN XIPHOPHORUS
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Southern blot hybridization with the viral oncogene probe v-src shows species- and genotype-specific banding patterns in DNA of Xiphophorus.
A 5.0 Kb band of X.maculatus can be related to an X-chromosomal copy of c-src. The hereditary melanoma that develops in X.maculatus/X.helleri/X.helleri backcross hybrids this copy becomes ampified in parallel with tumor progression. In melanomas that develop spontaneously in an X-irradiated laboratory strain c-src amplification is elevated up to 50 times over that of the controls. This elevation is in parallel with $pp60^{c\text{-}src}$ kinase activity.

INTERACTION BETWEEN WHITE AND BLACK MELANOCYTES AND KERATINOCYTES IN VITRO. Tomohisa Hirobe, Evelyn Flynn, and George Szabo. Laboratory of Electron Microscopy, Harvard School of Dental Medicine, Boston, MA.

Pure cultures of black (Negroid) or white (Caucasoid) melanocytes were co-cultured with white or black keratinocytes. The process of pigment donation and morphological changes in melanocytes was observed in vitro and by light and E.M. studies.

As expected, there was no genetic discrimination between the two cell types: white melanocytes donated melanosomes to black keratinocytes and vice versa. In co-cultures of white melanocytes and black keratinocytes an interesting effect was observed: white melanocytes located near black keratinocytes became more dendritic and more pigmented than white melanocytes cultured with white keratinocytes. No change in the morphology of black melanocytes was observed when co-cultured with white keratinocytes.

GROWTH CHARACTERISTICS OF HUMAN EPIDERMAL MELANOCYTES IN PURE CULTURE WITH SPECIAL REFERENCE TO GENETIC DIFFERENCES. Tomohisa Hirobe,* Evelyn Flynn,* George Szabo,* and Michael Vrabel.+ Laboratory of Electron Microscopy, Harvard School of Dental Medicine, Boston, MA* and the Human Nutrition Research Center on Aging at Tufts University, Boston, MA.+

Disaggregated epidermal cell suspensions derived from black (Negroid) and white (Caucasoid) foreskins were plated in medium which included cholera toxin and melanocyte growth factor (MGF) extracted from bovine hypothalamus. Initially 2% fetal bovine serum (FBS) was added to the culture medium. After 2 days the cells were transferred to serum-free medium to eliminate keratinocytes and fibroblasts. Preferentially the melanocytes began to grow, and after 12-16 days pure melanocyte populations could be harvested without keratinocytes and fibroblasts. Melanocytes were subcultured in the presence of 1% FBS; however, there was a striking difference in the growth rate and melanocyte yield of both primary cultures and subcultures despite the same inoculation density. The growth rate for black melanocytes was more than twice as fast as for white melanocytes; and the melanocyte yield from black skin was more than twice the yield from white skin. Black melanocytes proliferated even after the sixth passage; however, white melanocytes ceased to proliferate after the second passage, indicating that the MGF-dependent growth control mechanism in black melanocytes is different from that of white and suggesting genetic differences.

EFFECTS OF OXYGEN TENSIONS ON THE GROWTH AND PIGMENTATION OF CULTURED HUMAN MELANOCYTE.
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We have assessed the effect of oxygen tension on melanocyte growth, tyrosinase activity and melanin production. Melanocytes, seeded at 10^4 cells/cm^2 were grown in MEM with 5% FBS and 10 ng/ml 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Flasks were equilibrated with gas mixtures containing 5% CO_2 and various partial pressures of oxygen (PO_2, 7-620 mm Hg) and kept in incubators, electronically maintained at desired oxygen tensions. Melanocytes grew best at PO_2 6-34 mm Hg. Growth was reduced by 30% at PO_2 142 mm Hg, and even more at O_2 tensions greater than 230 mm Hg. 603 mm Hg was cytotoxic.

Tyrosinase activity (by the method of Pomerantz) was 300 μU/mg protein at PO_2 7-34 mm Hg. At PO_2 235 and 355 mm Hg, tyrosinase activity decreased to about 100 μU/mg protein. The apparent Km for tyrosine was unchanged in melanocytes cultured at various oxygen tensions. The Vmax, however, was decreased at the higher oxygen tensions (PO_2 235 mm Hg). At PO_2 between 6-135 mm Hg, the melanin content was propotional to tyrosinase activity. At cytostatic concentrations (PO_2 235 and 355 mm Hg), intracellular melanin content became some what higher, although tyrosinase activity itself was decreased. Low oxygen tension is favorable not only for melanocyte proliferation but also for tyrosinase activity.

DIFFERENTIATION AND TRANSDIFFERENTIATION OF AMPHIBIAN CHROMATOPHORES IN CLONAL CULTURE.
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The xanthophores and iridophores of bullfrog tadpoles transdifferentiate into melanophores in clonal culture (Ide, 1986). In the present study, the occurrence of the chromatophore transdifferentiation in vitro was examined using Xenopus laevis.

Neural crest cells migrated from neural tube explants were isolated and plated at clonal density in a medium conditioned with ventral tissues of Xenopus embryos. Three types of clonal colony appeared: (1) Melanophore colony, (2) Xanthophore colony and (3) Mixed colony composed of melanophores and xanthophores. The melanophores and xanthophores retained the ability of the formation of melanosomes and pterinosomes, respectively, indicating lack of transdifferentiation into melanophores in Xenopus xanthophores. Both the xanthophores appeared in the mixed colony and isolated directly from tadpoles proliferated without the transdifferentiation. However, iridophores of Xenopus embryos transdifferentiated into melanophores in clonal culture. The occurrence of the transdifferentiation depended on the chromatophores used and not on the culture medium.
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RNA-DEPENDENT DNA POLYMERASE (RDP) ACTIVITY IN MELANOMA OF XIPHOPHORUS
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RDP activity has been detected in muscle and melanoma. The activity bearing material has a density in sucrose gradient of 1.11-1.15 g/ml. Column chromatography on DEAE cellulose revealed elution of the bulk of the enzyme in the flow through. Further purification of the enzyme of the muscle by column chromatography on phosphocellulose revealed elution in the flow through and elution at 0.6 M KCl. The same procedure applied to the enzyme of the melanoma revealed elution in the flow through and at 0.1 M KCl. Melanoma contains probably an RDP which is different from that of the muscle. Both forms of RDP prefer poly(rC)p(dG)12-18 over other template primers. They are able to transcribe natural RNA as is excerted normally by retroviral RDP. - The activity of RDP in the melanoma is elevated up to 5 times over that of the muscle. This elevation is in parallel with the degree of malignancy of the melanoma, and with the degree of amplification and activity of oncogenes such as c-src and c-yes. We assume that RDP activity is related to oncogene amplification which might represent an important link in the chain leading to melanoma formation.

ULTRASONIC CHARACTERIZATION OF MELANOSOMES IN B16 AND HARDING PASSEY MELANOMAS DOPED WITH 4-S-CYSTEINYL PHENOL: Ryusuke Kono and Kowichi Jimbow*
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Most atempts to develop rational approach for diagnosis and/or treatment of malignant melanoma have involved direct or indirect exploitation of various steps of melanin synthesis. In the previous report (Jimbow et al., J. Invest. Dermatol., 84: 355, 1985 abstract), it was shown that 4-S-cysteinyl phenol (4-S-CP) which is made by combining phenol with cysteine through thioether bond, is the substrate of mammalian tyrosinase and selectively incorporated into melanoma tissure. This study reports the ultrasonic measurement of the melanosomes which were isolated from B16 and Harding Passey (HP) mouse melanoma and which were incubated in vitro with 4-S-CP. We found (a) that HP melanin possesses an amorphous structure at a dry state and a linearly ordered structure in water suspensions buffered at pH 6.8, (b) that B16 melanin exhibits the orderedstructure in the both dry and water-suspended states, and (c) that a doped of 4-S-CP tends to suppress the melanization of melanosomes, probably through interaction with tyrosinase. Our ultrasonic measurement suggests that 4-S-CP is directly incorporated into the melanosomes, hence it becoming cytotoxic through the polymerization within the melanosomes.

TRANSFORMATION OF HUMAN MELANOCYTES IN VITRO
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Normal human melanocytes grown in vitro in the presence of 12-myristate phorbol 13-acetate (PMA, 10 ng/ml) and cholera toxin (CH.T.10^{-8}M) were shown to retain their differentiated phenotype, normal diploid karyotype (for 10-15 passages) and their growth in vitro to be TPA dependent. Addition of isobytylmethylxanthine (IBMX 10^{-4}M) to such cultures furthered melanocyte growth (1). Six to eight weeks of continuous growth of melanocytes in TPA, CH.T. and IBMX (melanocytes previously grown in TPA and CH.T. for 15-20 passages) resulted in marked morphological changes. Morphologically changed cells grew into colonies (5-7 colonies/5-7x10^5 melanocytes) which were isolated and shown to be transformed. The transformed cells had morphological features resembling melanoma cells, expressed melanocyte surface markers and surface markers typical of melanoma cells. They could grow independent of TPA and IBMX and formed colonies in soft agar. Karyotyping revealed these cells to be aneuploid with multiple copies of chromosome #7. Two different melanocyte cell strains were so far transformed, and three are presently being studied.
The described system offers an opportunity for detailed studies of melanocyte transformation into a melanoma-like cell.

Reference:
1. Halaban R, Alfano FD. In Vitro 20:447,1984.

PIGMENT CELL TUMORS AND SCHWANNOMAS IN FISH

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Pigment cells and Schwann cells are considered to be of neural crest origin. In teleosts, tumors originating from these cells are the most frequent. We collected erythrophoromas, red pigment cell tumors (36 goldfish and 14 multicolored carp), melanomas (27 nibe croakers, 1 bitterling and 4 medaka), and schwannomas (23 coho salmon, 1 African lungfish and 1 sea bass). Histopathological findings in these tumors are described. Although in humans pigment cell neoplasms and schwannomas are easily distinguishable, in fish pigment cell elements and Schwann cell elements in tumors are sometimes mixed making differential diagnosis difficult. Therefore, we approached this problem in terms of ultrastructural and immunological investigations. Demonstration of specific pigment organelles, such as melanosomes, pterinosomes, and reflecting platelets in the cells by electron microscopy was found to be the most reliable for diagnosis of pigment cell neoplasms. Immunohistochemical staining was also useful in diagnosis of neural crest tumors in fish. On immunohistochemical staining, all the erythrophoromas in goldfish and some of the schwannomas gave a positive reaction for S-100, which is usually used in diagnosis of human neurogenic tumors. These facts suggest that neural crest tumors in fish can be diagnosed by much the same criteria as neuronal tumors in humans.

THE CATALYTIC EFFECT OF L-DOPA-MELANIN ON THE TYROSINASE-MEDIATED OXYGENATION OF PHENOLIC DEPIGMENTERS

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The melanocytotoxic phenols, p-t-butylphenol and p-hydroxyanisole are substrates for mushroom tyrosinase. Added synthetic L-DOPA melanin greatly accelerates the formation of the respective 1,2-quinones in a model in vitro system. Studies with C57-BL mice indicate that these quinones are cytotoxic in vivo. Kinetic experiments with synthetic ("unoxidized") melanin and with ferricyanide-oxidized melanin indicate that the pigment most likely accelerates the reaction by acting as a DOPA-like cofactor. For p-hydroxyanisole, but not for p-t-butylphenol, anerobic electron transfer reactions from phenol to melanin are also important. We feel that this is an interesting illustration of melanin's "dark side", in which it may facilitate harmful reactions, instead of acting as a protective agent. Supported by Projects #1-R01-OH-01556 from NIOSH and #812605-01 from the Environmental Protection Agency.

FURTHER STUDIES ON MULTIPLE DIFFERENTIATION OF GOLDFISH ERYTHROPHOROMA CELLS IN VITRO

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Permanent cell lines, named GEM, were establsihed from spontaneous tumors of erythrophores (erythrophoromas) appearing in goldfish. These cells expressed a variety of phenotypes in vitro upon chemical induction of differentiation. Observed phenotypes were classified into four categories based on their representative characters: (1) pigmentation such as pterinogenesis, melanogenesis and genesis of reflecting platelets, (2) formation of dermal skeletons such as teeth and scales, (3) extension of long dendrites like neurons and (4) lens-like structures.

In the present study, we report (1) clonal heterogeneity of melanosome ultrastructures produced by melanogenic variants and (2) immunocytochemical characterization of neuronal properties present in neuron-like dendritic cells and (3) inducibility of these cell characters form a stem cell type clone of GEM cells. Possible reasons for restrictions of phenotypic expression of induced GEM cells to cell characters of neural crest origin are discussed.

ROLE OF CELL SURFACE GALACTOSE AND GALACTOSIDE-BINDING LECTIN IN CELL-CELL AND CELL-SUBSTRATUM ADHESION OF XENOPUS LAEVIS MELANOPHORES. N.C. Milos, H.C. Wilson, T.M. Mohanraj and G.E. Shand, Dept. of Anatomy, University of Alberta, Edmonton, Alta., Canada. T6G 2H7.

Neural crest of X. laevis grown in stationary culture produces unpigmented migratory cells that develop into melanophores (MEL). Later, MEL cease active migration and make contact via cytoplasmic extensions suggesting some degree of mutual cell adhesiveness. MEL utilize cell surface galactose (GAL) in cell-substratum adhesion (J.E.Z., in press). In the present work, cultures of MEL were trypsinized to obtain single cell suspensions. Cells were aggregated in complete saline using a rotatory shaker. Under these conditions, MEL were mutually self adhesive, forming smooth cohesive aggregates by 1 h. Addition of jack bean β-galactosidase to remove cell surface GAL correlated with decreased MEL-MEL adhesion. Galactoside-binding lectin activity is detectable in these cultures. Addition of thiodigalactoside (TDG-10 mM-a potent lectin inhibitor) to aggregating cells was correlated with decreased MEL cell-cell adhesion. Loose cell clusters formed. Embryonic lectin activity was purified on a column of p-aminophenyl-β-D-thiogalactoside. In preliminary work, addition of lectin or TDG to stationary cultures at the stage of cell migration was correlated with changes in MEL morphology and more regular MEL-MEL cell spacing. These results suggest that cell surface GAL and endogenous galactoside-binding lectin activity play a role in MEL adhesion. Support: AHFMR and MRC of Canada.

OBSERVATIONS ON THE POSITION OF MELANOCYTES WITH RESPECT TO THE EPIDERMAL BASEMENT MEMBRANE. Gisela E. Moellmann, Elizabeth Kuklinska, Sidney N. Klaus and Aaron B. Lerner, Yale University School of Medicine, New Haven, CT, USA.

Epidermal melanocytes are usually integrated into the basal layer of keratinocytes and rest on a modified basement membrane that has an attenuated lamina lucida, lacks anchoring fibrils and resembles endothelial basement membrane or the basement membranes of smooth muscle and Schwann cells rather than epidermis. Basal Langerhans cells, on the other hand, do not rest on a basement membrane but are cushioned by a thin sheet of keratinocyte under which the basement membrane is typically epidermal. These cells are mobile and of recent subepidermal origin.

We report here on several clinical, developmental and experimental conditions in which melanocytes, too, are located in parabasal positions: autologous cultured melanocytes injected into a suction blister in order to populate a piebald area; melanocytes in a case of generalized epidermal melanosis in late-stage metastatic melanoma; melanocytes in repigmenting patches of vitiligo; melanocytes in freckles and in hyperpigmented macules bordering on piebald epidermis; melanocytes of an infant (3 mos) with ocular albinism; and melanocytes of an epidermal graft established from autologous cultures of epidermis and applied over a dermal allograft.

A common denominator in these conditions may be mobility of melanocytes within the basal layer of epidermal cells in an attempt to establish a balanced network of epidermal melanin units.

ULTRASTRUCTURAL STUDY ON MELANOGENESIS OF FOLLICULAR MELANOCYTES IN THE NEW BORN BEIGE MICE

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The beige trait (bg/bg) in mice closely resembles the Chédiak-Higashi syndrome and this mutation affects pigment granules of melanocytes, resulting in the formation of abnormally large and irregular melanin granules. These melanin granules have been thought to arise from successive fusions of primary lysosomes with melanin granules which are already enlarged from multiple fusions among melanosomes. Although the morphological characteristics of the giant melanin granules have been adequately described previously, the early melanosome development remains unknown. Electron microscopic studies were conducted in the differentiating follicular melanocytes of the new born beige mice to determine the early development of melanosomes and the mechanisms of fusions among melanosomes. Our findings included; (1) melanosomes at each stage found within the melanocytes during early stages of hair growth of 1-3 day - old mice were essentially similar in size and shape to those of C57BL/6J mice.; (2) the rate of fusions among melanosomes tended to be increased as the hairs grew. These findings suggest that during the early differentiation process of follicular melanocytes, all stages of melanosome development are not essentially affected by the beige gene, and subsequent fusions among melano-somes at the late differentiation process may be responsible for the early formation of the giant melanin granules of the beige mice.

A NOVEL MODEL FOR TESTING ENHANCERS OF PIGMENTATION. Warwick L. Morison, Antoinette F. Hood, Robert M. Sayre, and Patricia Poh Agin. Dept. of Dermatology, Johns Hopkins University, Baltimore, MD 21204 and Dept. of Photobiology Research, Plough, Inc., Memphis, TN 38151.

Mouse skin, unlike human skin, does not contain active epidermal melanocytes with the exception of the ear and tail skin in some pigmented strains. We have investigated enhancement of pigmentation in inbred C3H mice using these two areas of skin as a model for testing the effects of phosphorylated DOPA (DP) and ultraviolet light. Mice were restrained by cage dividers and treated with various doses of DP in DMSO or DMSO alone with or without the addition of UV radiation (30 min. exposure, 3x wk. for 4 weeks to Kodacel-filtered FS-40 lamps). Each tail served as its own control since only the dorsal surface was treated and irradiated. Pigmentation was graded blindly on histologic sections stained with Fontana-Masson. UV radiation caused a marked increase in epidermal and dermal pigmentation and this was enhanced in a dose-dependent manner by 0 to 0.05% DP. However, there was minimal effect from DP alone.

A MELANOCYTE DIFFERENTIATION ANTIGEN RECOGNIZED BY A NEW MURINE MONOCLONAL ANTIBODY.

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The murine monoclonal antibody (MoAb) 2G10 (IgG1) has been obtained by immunization of Balb/c mice with freshly explanted human melanoma cells mixed with Bordetella Pertussis adjuvant. The MoAb which recognizes on melanoma cells a 76Kd single chain polypeptide has been extensively tested immunohistochemically on a variety of human normal and tumor tissues. The 76Kd molecule appears to be expressed on fetal (8 weeks) and adult epidermal and choroidal melanocytes, but undetectable on other normal fetal and adult tissues of different histotype as well as in non melanocytic tumors. The antigen on the other hand is present in type A cells of nevocytic nevi, in congenital nevi, in blue nevi, and with various frequency in primary and metastatic melanomas including choroidal tumors. These data indicate that MoAb 2G10 identifies a novel melanoma associated antigen which represents a marker of early differentiation in the melanocyte cell lineage.

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GENETICS OF ALBINOS IN JAPANESE POND-FROGS.
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In the Rana nigromaculata group, a total of 13 albino stocks have been obtained by this time. While three of them were produced by irradiating gametes, the others were collected from the field. By mating experiments and diploid gynogenesis, it was found that the albinos of each stock are mutants due to a single recessive gene in the homozygous condition. Females of each of the 13 albino stocks were mated with males of the other 12 stocks. The results showed that the 13 albino stocks can be divided into five groups. While matings between different albino stocks of the same group produce albinos only, those between albinos of different groups produce wild-type individuals alone. Thus, it is evident that the albinos of each group have a locus differing from the loci of the other groups. Only the first group is divided into three strains controlled by multiple alleles, and one of the strains contains six stocks including the three induced by irradiation of gametes. Although all the albinos are generally light yellow in dorsal surface and red in pupils, different groups or strains slightly differ from one another in these colors.

A genetic map showing the chromosomes bearing the albino genes and the positions of the loci on the respective chromosomes was established. These findings were obtained by comparing the genotypes and the constitution of each of the bivalents in each oocyte in female backcrosses produced from female hybrids between Rana nigromaculata and R. brevipoda by utilizing albinic males.

MORPHOLOGICAL EVIDENCE FOR STIMULATION OF MELANOGENESIS BY LYSOSOMOTROPIC AGENTS AND MONENSIN IN CULTURED B16 MELANOMA CELLS
A. Oikawa, H. Saeki, T. Akiyama and J. Matsumoto. Tohoku University, Sendai and Keio University, Yokohama, Japan

The population densities of pigmented and unpigmented melanosomes were determined under a transmission electron microscope as numbers of vesicles per unit area of cytoplasmic cross-sections of 20 to 40 cells. When the degree of melanosome maturation of a cell was defined as the ratio of pigmented melanosomes to the sum of pigmented and unpigmented melanosomes on the cross-sectional unit area in the cell, the maturation was significantly stimulated by chloroquine or monensin, without any change in the sum of two types of melanosomes. The heaviest melanization was observed in NH_4Cl-treated cells and this was due to an increase in the total number of melanosomes in addition to the maturation of immature melanosomes.

These observations are consistent with the reported biochemical results (J. Invest. Dermatol. *85*:423, 1985) that monensin instantaneously stimulates the tyrosinase activity of these melanoma cells in culture and that lysosomotropic agents stimulate not only the tyrosinase activity but also stimulate the *de novo* synthesis of the enzyme.

EVALUATION OF RELATIVE LEVELS OF HLA-DP, DQ AND DR TRANSCRIPTS AS MOLECULAR MARKERS FOR MALIGNANT TRANSFORMATION OF MELANOMA CELLS: CORRELATION WITH CLINICAL FEATURES. Magali ROUX-DOSSETO, Françoise ROUGE, Georges TOCCO, Charles Le BORGNE de KAOUEL and Christian AUBERT. U119-INSERM 27, Bd Leï Roure 13009 Marseille, FRANCE

Human class II antigens are encoded by the D region of the HLA complex. Recently, cloning technics allowed the molecular analysis of the HLA-DP (1), -DQ (2) and -DR (3) subregions. The corresponding antigens have limited expression on normal cells. However, they have been found on tumor cells, specially on melanoma cells. The overall levels of class II antigens expressed on melanocyte cell surface have been determined using serological approaches and wide variations have been found. Such variability did not account for respective expression of DP, DQ and DR antigens, as most of antibodies directed against DR molecules also reacted wih DP and DQ antigens (4). Using statistical comparisons, we identified locus specific sequences within DP, DQ and DR encoding cDNAs (5). This allowed us to prepare specific probes for DP, DQ and DR loci.
In the present report, we investigated the expression of human class II antigens in melanoma cells in correlation with clinical features of the disease as stage invasion, growth rate and metastatic behaviour. Total cytoplasmic RNA was prepared from well characterized melanoma cell lines (6). Serial dilutions were dotted onto nitrocellulose sheets and hybridized with probes for DP, DQ and DR loci. The relative levels of corresponding transcripts in melanoma cells have been compared to those found in dyplastic, junction and benign nevi cells in order to evaluate the usefulness of class II transcripts as molecular markers for malignant transformation.

GENE EXPRESSION IN HUMAN MALIGNANT MELANOCYTES : MOLECULAR CLONING OF SEQUENCES CODING FOR RARE MESSAGES. Magali ROUX-DOSSETO, Georges TOCCO, Françoise ROUGE and Christian AUBERT. U119 - INSERM, 27 Bd Leï Roure 13009 Marseille, FRANCE

A modified cloning method designed to produce differential complementary DNA libraries permits the isolation of sequences that are present in the RNA population of any development stage or tissue, but are not present or are much less abundant in another stage or tissue. Selective complementary DNA cloning is especially useful when the differentially expressed RNAs are too low to moderate abundance in the cells in which they occur.
Previously, we reported that cell cultures of human primary melanoma tumors go through a dedifferentiation phase before showing again the differentiated characteristics of the original melanocytes (C. Aubert et al., J. Natl. Cancer Inst., 1977, 58, 29). Over 200 cDNA recombinants clones homologous to polyadenylated RNA which are significantly more prevalent in malignant melanocytes than in fetal fibroblasts have been analyzed by colony hybridization with 32P-cDNA prepared from several stages of melanocytes differentiation. A class of polyadenylated RNAs differentially expressed in redifferentiated malignant melanocytes have been isolated. These melanoma cell RNAs occur very rarely or not in dedifferentiated - non malignant- melanocytes, accumulate as the result of transcription in melanoma cells. These RNAs may encode sequences related to the malignant phenotype.

THE ROLE OF THIOREDOXIN REDUCTASE IN REGULATING MELANIN BIOSYNTHESIS AND ITS CONTROL BY CALCIUM. K.U. Schallreuter, J.M. Wood, and M.R. Pittelkow*. Departments of Dermatology and Biochemistry, University of Minnesota and Mayo Clinic, Rochester, MN*

A membrane associated thioredoxin reductase has been purified by affinity column chromatography from cell cultures of human keratinocytes. The enzyme has been shown to be effective in the reduction of free radicals on the outer plasma membrane of the epidermis both in vivo and in vitro. A correlation has been established between the reduction of free radicals by this reductase and the regulation of melanin biosynthesis. Decreased enzyme activity was found in depigmented skin of vitiligo, compared to normal pigmented skin of the same donor. Keratinocyte cell cultures from vitiligenous and normal skin established from the same individual revealed a three-fold reduced activity (rates/10 min utes) in the presence of 2 mM Ca^{2+}, compared to cells from normal healthy controls. It has been shown in vivo on guinea pig skin, in vitro on cell cultures of human keratinocytes, and on the purified enzyme itself that the extracellular free calcium concentration regulates the activity. The possible role of calcium ions in the etiology of vitiligo will be discussed.

SIMILAR GROWTH RESPONSES TO INSULIN BY MELANOMA CELLS FROM SEPARATE MAMMALIAN SPECIES. Andrzej Słominski, Tessie McNeely, John McLane, and John Pawelek, Department of Dermatology, Yale University School of Medicine, New Haven, CT, USA, and Andrzej Bomirski, Department of Biology and Genetics, Gdansk Medical School, Gdansk, Poland.

Studies with Cloudman S91 mouse melanoma cells in culture have shown that insulin is a growth factor for these cells, but that the effects can be either inhibitory of stimulatory depending on growth conditions and cellular genotype. We were interested to determine whether the inhibitory action of insulin on Cloudman cells was a phenomenon unique to descendents of this particular melanoma tumor, or whether growth inhibition by insulin might be a characteristic of mammalian melanoma cells in general. In this report we show that cells from another melanoma, the Bomirski hamster melanoma, are also inhibited in their proliferation by insulin. The effects of insulin on the Bomirski cells are similar to those on the Cloudman cells in that insulin can stimulate as well as inhibit Bomirski cells, depending on growth conditions. In addition, Bomirski cells responsive to insulin express the ability to phosphorylate a protein with a molecular weight of somewhat less than 90K daltons. This protein has characteristics similar to a phosphoprotein recently observed in insulin-responsive Cloudman melanoma cells. Our results suggest that growth regulation by insulin is potentially an important characteristic of mammalian melanoma cells, and further that the phosphorylated state of a specific protein is a likely determinant of cellular responsiveness to insulin.

MOLECULAR GENETIC ASPECTS OF MELANOMA FORMATION IN XIPHOPHORUS
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In order to contribute to an understanding of the molecular biological basis of melanoma formation, we are on the one hand attempting to isolate and characterize the oncogene Tu from Xiphophorus, whose deregulation has been proposed to lead to melanoma formation (Anders et al., Adv. Canc. Res. 42; 191, 1984), by progressive subdivision and sib-selection of a cosmid library using a DNA transfection bioassay for Tu. On the other hand we are analyzing the structure and expression of known proto-oncogenes in various mutants of Xiphophorus affecting pigment cell differentiation and melanoma formation. For a cloned Xiphophorus proto-oncogene from the src-related members of the tyrosine kinase family we could show differential expression during normogenesis, overexpression in melanoma, and on the protein level a correlation in expression to Tu. The nature of that correlation, which does not comprise structural identity, is further analyzed. - Some other known proto-oncogenes were also found to be expressed in malignant melanoma as well as in a melanoma derived cell line. This suggests to us that other genes in addition to Tu might be involved in the induction and/or progression of the melanoma.

2-S-CYSTEINYLHYDROQUINONE SPECIFICALLY INHIBITS CELL-FREE PROTEIN SYNTHESIS OF HARDING-PASSEY MOUSE MELANOMA BY BLOCKING AMINOACYL TRANSFER RNA FORMATION.
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During the survey work about the effect of sulfur-containing tyrosine analogs synthesized chemically on the melanoma melanocyte-specific protein synthesis, we found that 2-S-cysteinylhydroquinone (2-S-CHQ) specifically inhibits the cell-free protein synthesis by microsomes and cytosol prepared from Harding-Passey (H-P) mouse melanoma. Analogous cell-free protein synthesizing systems from rat liver, rat AH130 hepatoma, rat intestinal tumor (Sasa B-1), and B-16 mouse melanoma were not inhibited. To elucidate the cause of this H-P melanoma-specific inhibition, we examined the inhibitory effect of 2-S-CHQ on the limited reactions of cell-free protein synthesis from H-P melanoma, and found that aminoacyl-tRNA formation step is the site of the inhibition. Since the activity of aminoacyl-tRNA synthetases from H-P melanoma was not affected by 2-S-CHQ, it was suggested that tRNAs from H-P melanoma interact with 2-S-CHQ. Analysis of the charging activities of each tRNA from H-P melanoma revealed that the charging of several amino acids, such as Leu, Gly, Phe, Ala, Tyr, Met and Ile, was inhibited by 2-S-CHQ. These results suggest that some of H-P melanoma tRNAs have different structures from those of rat liver, rat hepatoma, and B-16 mouse melanoma.

ISOLATION OF XANTHOBLASTS FROM THE DERMAL TISSUE OF GOLDFISH AND THEIR DIFFERENIATION BY dbcAMP OR ACTH AND CARP SERUM IN CULTURE.

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To identify blasts of fish xanthophores, non-pigmented cells without any phenotypic traits as pigment cells were isolated from the dermal tissue of xanthic goldfish and cultured in a medium containing 1 mM dbcAMP or 0.25U/ml ACTH and 10% carp serum. These non-pigmented cells changed their morphology to dendritic shape and showed yellow pigmentation in the cytoplasm during a few days. Paper chromatography of pteridines and electron microscopic studies revealed that the yellow-colored cells had a high level of sepiapterin as a main component of pteridines and many young pterinosomes with inclusions of fuzzy materials. These characteristics of the yellow-colored cells coincide with those of larval xanthophores of goldfish. These results suggest that non-pigmented cells isolated in the present study are possibly xanthoblasts.

PROLIFERATION OF PIGMENT CELLS FROM TOP-MINNOW, GAMBUSIA AFFINIS. M. Yasutomi, Biol. Lab., Aichi Medical Univ., Aichi, Japan 480-11.

In order to get pigment cell line from fish pigment cells of some fish were cultured. Among them iridophores of G. affinis proliferated successfully and cell culture of its melanophores was partially successful. This fish is ovoviviparous, so we can get fetuses sterilely from its uterus. The fetuses were minced by scissors and cultured in a Falcon dish at 25°C. As culture medium 199 medium containing 10% fetal calf serum, 2% carp serum and antibiotics was used. Fibroblasts migrated actively from the explants and then iridophores and melanophores came to migrate on the fibroblasts. For subculture of pigment cells 199 medium was exchanged by Ca, Mg free Hanks solution containing 0.5% trypsin and the dishes were incubated for 20 min. Obtained pigment cells and fibroblasts were cultured. The fibroblasts proliferated rapidly and covered the bottom of the dish. Proliferation of iridophores and melanophores occurred on the fibroblasts 3-5 weeks after inoculation. Doubling time of iridophores was about 5 days and their number in one colony attained to over 5000 cells. But melanophores colony comprised only about 50 cells and did not show further proliferation.

DOPACHROME CONVERTING ACTIVITY IN HAIR FOLLICLES OF Phodopus sungorus. Brian Weatherhead, Department of Anatomy, University of Hong Kong, Hong Kong.

During the spring and autumn moults of the Djungarian or Siberian hamster the tyrosinase levels in the hair follicles increase. However, at the autumn moult the winter coat that grows is unpigmented despite these elevated levels of tyrosinase, suggesting a post-tyrosinase inhibition of melanogenesis. Using the assay method described by Barber et al (J. Invest. Dermatol. 83:145-149, 1948), I have shown that the ability of the hair follicles of Phodopus to convert dopachrome is photoperiodically modifiable being reduced in those animals growing a white coat under short day photoperiods (8L:16D) compared to non-moulting animals kept in long day photoperiods (16L:8D). This reduced ability to convert dopachrome may be in part responsible for the failure of hair follicles with high levels of tyrosinase to produce pigmented hair.

MOLECULAR APPROACH TO STUDY MELANOMA FORMATION IN XIPHOPHORUS

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In Xiphophorus, melanoma may develop following interracial and interspecific hybridization. Neoplastic transformation of the pigment cells is in both cases mediated by an genetically identified oncogene designated as Tu. Expression of Tu is under control of regulatory gene systems composed of several linked and nonlinked genes.

Besides Tu cellular homologues of several viral oncogenes have been identified in the genome of Xiphophorus. Out of these oncogenes c-sis and c-erb were studied in more detail. In contrast, c-erb of X.helleri is differently organized compaired to that of X.maculatus. Both, c-erb and c-sis were isolated from a Xiphophorus EMBL 4 gene-library and characterized by restriction mapping and sequencing. We are now trying to assign c-erb to chromosomes and melanoma formation.

Development-Genetics-Cell Biology

ANALYSIS AND QUANTIFICATION OF MELANINS: AN IMMUNOLOGICAL APPROACH

Ago B. Ahene, Walter H. Koch, Walter Maldonado, and Miles R. Chedekel
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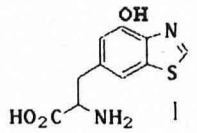
The best current procedures for analysis and quantification of melanins involve chemical degradation followed by HPLC analysis. In attempts to lower the level of sensitivity we have been investigating alternative techniques. HI hydrolysis of pheomelanin affords a well characterized set of degradation products among which is the hydroxybenzothizole amino acid I. This degradation product arises from breakdown and rearangement of the primary pheomelanin chromophore, and accounts for over 10% of the weight of pheomelanin prepared by the enzymatic polymerization of 5-S-cysteinyldopa. Indeed, identification of I following HI hydrolysis is taken as proof of the presence of pheomelanin in melanotic tissue



I

Compound I was synthesized, coupled to bovine serum albumin, and subsequently injected subcutaneously into New Zealand White rabbits. A standard protocol designed to produce antibodies against I was employed, and a radioimmunoassay capable of measuring subnanogram levels of I in model melanins and melanogenic tissues was developed from the resultant antiserum. The assay characteristics and applications will be discussed

PHOTOCHEMICAL PROPERTIES OF PHEO- AND EU-MELANINS.

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The effect of light on the stability of pheo- and eu-melanins both in their native milieu (human hair) and in vitro has been studied using full spectrum sunlight as well as specific wavelength (300, 350 and 450 nm) exposure. The course of the reaction was followed by reflectance spectroscopy in the case of hair and by UV-visible and differential spectroscopy in the case of melanin solutions.

The response of native melanins to light exposure paralleled the behavior of those in vitro. Irradiation of solutions of melanins in the presence of oxygen at pH 7.4 caused a continuous decrease in absorbance of eumelanin while bringing about initially an increase in the absorbance of pheomelanin. In this case, the differential spectra point to the build-up of a chromophore at 369 nm with a concommitant absorbance decrease at 254 nm. At higher pH values, both melanins photobleach rapidly.

^{13}C N.M.R. STUDY OF MELANINS

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The commercial availability of n.m.r. spectrometers operating in CP-MAS (Cross Polarization Magic Angle Spinning) has provided a powerful tool to the investigation of insoluble materials.

We have undertaken a C-13 n.m.r. study of melanins under high resolution conditions using a Jeol GX-270 spectrometer equipped with a Chemagnetics solid state accessory. The samples (0.5 g in Delrin rotors) are spinned at $\sim$ 3.5 kHz and spectra with reasonably good S/N ratio are collected in few hours. The spectra clearly show absorption regions for sp^3 and sp^2 carbons and peaks corresponding to different chemical functions can be assigned.

The spectral features of different melanin samples are compared and discussed.

COLLISIONAL SPECTROSCOPY IN STRUCTURAL CHARACTERIZATION OF MELANINS.- 1.THE COLLISIONAL MASS SPECTROMETRIC BEHAVIOUR OF MELANIN PRECURSORS.

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B.Pelli and P.Traldi, CNR, Area di Ricerca di Padova - Italy.

In the attempt to obtain a structural characterization of tryptophan derivative melanins obtained either by synthetic or by biosynthetic pathways, we have thought of interest the use of modern mass spectrometry.
Looking at the exciting results obtained by such technique, in particular by collisional spectroscopy, in the characterization of compounds in complex matrices, we have undertaken the present study to investigate the mass spectrometric behaviour of the main tryptophan melanin precursors, i.e. indole derivatives and kynurenines, in comparison with that of physiological precursors, like tyrosine and DOPA. This preliminary work results essential for the determination of such molecules in the terribly complex mixture arising from the pyrolysis of the melanins themselves.

CARBON-13 NMR SPECTROSCOPY AS A TOOL FOR STRUCTURE ILLUCIDATION OF MELANINS.

Purshotam Bhan*, Kanury V. Subbarao*, Thomas M. Shultz**, and Miles R. Chedekel*
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The tyrosinase catalyzed polymerization of 5-S-cysteinyldopa (5SCD), affords a homogeneous chromophore which serves as an excellent model for biosynthetic and structural studies on pheomelanins. 5SCDs specifically labeled (> 99% enrichment) with carbon-13 on both the alanyl and cysteinyl side chains were synthesized and subsequently converted to pheomelanins by the action of mushroom tyrosinase. Only signals originating from the labeled carbons are observable in the high resolution carbon-13 NMR spectra of the resultant pheomelanins. Single frequency off resonance decoupling experiments and carbon-13 chemical shift values allow us to gain information on the number of attached protons and hybridization states of the observed carbon resonances. These studies introduce a new and superior methodology for examining the structure and homogeneity of melanin pigments as well as provide a tool for evaluating the effects of various isolation, purification, and storage protocols on melanin stucture Results from these studies will be discussed.

APPLICATIONS OF SURFACE SCIENCE TECHNIQUES FOR FUNCTIONAL GROUP ANALYSIS OF EUMELANINS.

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One of the most difficult questions regarding the chemistry of eumelanins involves the initial preparation of the sample for analysis. Standard methods have centered on the alkaline treatment of the material followed by collection of the now water soluble eumelanin. The severity of alteration of the original polymer is a crucial parameter in trying to extrapolate information gathered on the solubilized eumelanin back to the intact and unperturbed system. We now report the results of ESCA analyses on eumelanins before and after solubilization. Standard samples of sepia, 5,6-dihydroxyindole (DHI), and L-DOPA derived eumelanins were solubilized with alkaline H_2O_2[1] and the acid insoluble portion (Melanin Free Acid, MFA) retained. Examination of specific functional groups shows that the solubilization process creates MFAs that are very similar in C=O and C=N content.

Comparison with melanins generated from derivatives of DHI indicate that imino and pyrrole carboxylic acid groups constitute a large portion of this functional group distribution.

1. L.J. Wolfram, Solubilization of Eumelanins, VI European Workshop on Melanin Pigmentation, Sept. 22-25, 1985, Murcia, Spain.

X-RAY DIFFRACTION STUDIES ON MELANINS AND MELANOSOMES

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A systematic work of analysis and comparison of X-ray diffraction spectra has been performed on melanins of various kinds and origin. A careful study of synthetic melanin from L-dopa was performed on powder samples using Cu Kα radiation (λ=5417Å) in order to determine the details of the scattering intensity at small Q values ($Q < 6$ Å^{-1}). The results showed that the technique can allow to obtain informations that in the past, when obtained, were disregarded.

The analysis on synthetic melanin was employed as a starting point to examine natural samples: melanins and melanosomes from equine eyes and melanoma, and melanosomes from embryos of Bufo-Bufo.

All the spectra show rather sharp peaks (the three main at Q=1.43 Å^{-1}, Q=2.6 Å^{-1}, Q=5 Å^{-1}) superimposed on an amorphous background, typical of paracrystalline systems. A Fourier inversion of the spectra has been performed, by standard methods, in order to extract a radial distribution function g(r). The g(r) obtained are compared and discussed.

MELANIN FREE ACID - A CHEMICAL STANDARD FOR EUMELANIN RESEARCH

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Although chemical endeavors into the structure and reactivity of eumelanins have been extensive, diseparate and irreproducible results from different laboratories has led to a certain amount of confusion in the field. We now offer a chemical standard for eumelanin research. Eumelanins generated from synthetic L-DOPA specifically enriched with C-13 at the benzylic position (A) and from 5,6-dihydroxyindole labeled at carbon 2 (B) were used as the precursors. The materials were converted enzymatically and auto-oxidatively to the solid polymers and then converted to their water soluble form by solubilization with alkaline H_2O_2. Reproducible mass balances are obtained along each pathway. The chemically modified materials (melanin free acids; MFAs) were then characterized by FT-IR difference spectroscopy, C-13 NMR, ESCA, and chromatographic analyses.

The L-DOPA derived MFA has C-13 NMR signals at 38 and 110 ppm being due to the carbons in A and B. The DHI derived MFA, however, only shows a signal at 110 ppm. IR information shows that much of the carbonyl character comes from an aromatic carboxylic acid. It is apparent that the L-DOPA MFA generated auto-oxidatively more closely resembles DHI-MFA and, hence, is an excellent marker for eumelanin investigation.

QUINONE OXIDOREDUCTASE IN PORCINE RETINAL PIGMENT EPITHELIAL (RPE) CELLS IN VITRO.
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Melanogenesis in the RPE is restricted to the embryonal and neonatal periods. However, the adult cells do exhibit a low level of tyrosinase and when placed in tissue culture, they release into the medium several melanogens. Since the RPE is located in an oxygen rich environment subjected to constant illumination, the potential toxicity of the quinone intermediates would be greatly enhanced by the catalytic generation of superoxide by semiquinones under these conditions. We therefore considered possible cellular mechanisms for limiting the exposure to quinones through regulation and detoxification.

We have demonstrated that the RPE cells contain both dopachrome conversion factor and significant activity of the detoxifying enzyme quinone oxidoreductase (E.C. 1.6.99.2; DT-diaphorase). It is distinquished from others by specific inhibition at 10^{-8} M dicoumarol and by its lack of specificity for pyridine nucleotide electron donors. Since the quinone oxidoreductase converts quinones to dihydroxyquinones, we hypothesize that it may also act as a regulator of melanogenesis.
Supported by EY0496 from NEI.

DEMONSTRATION BY PYROLYSIS-GLC OF AN EFFECT OF TREATMENT WITH ACID OR UREA ON MELANIN
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Established methods for the isolation of melanin from biological tissues use both hydrochloric acid digestion and treatment with urea to separate melanin from other material. This study provides evidence that such treatments can lead to a change in melanin structure. Synthetic-tyrosine derived melanin (Sigma Chemical Company) was subjected to either acid digestion (6M hydrochloric acid at 110°C for 72h) or treatment with 8M urea for 24h followed by washing with dichloromethane: methanol 1:1. The treated melanin was collected by centrifugation and both treated and untreated melanin were washed several times with distilled water. The melanin samples were then dried and subjected to pyrolysis-GLC under the following conditions: 500µg of melanin was pyrolysed on a platinum ribbon filament of a Pyroprobe (CDS). The pyrolysate was chromatographed on a bonded phase SE30 fused silica column with an oven temperature program and FID detection.
While visual inspection of the pyrograms could yield little information, analysis of the pyrograms by means of principal component analysis and linear discriminant analysis showed that acid treatment produced a marked effect on the pyrogram. The effect of the urea treatment was less marked, nevertheless, this effect was significant. While it is not possible to make deductions about the structural changes that have occured, the technique provides unequivocal evidence that some change has taken place.

THE EFFECT OF HYDROQUINONE TREATMENT ON THE CONTENT OF 5-S-CYSTEINYLDOPA IN GUINEA PIG SKIN.
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To determine whether the depigmenting agent hydroquinone (HQ) influences the level of the phaeomelanin precursor 5-S-cysteinyldopa (5SCD), wax epilated skin on the flanks of eight black guinea pigs was treated daily with 4% HQ solution for periods of two and four weeks. Some areas of skin were treated with the vehicle alone to act as a control. Skin samples were homogenised in trichloracetic acid and the supernatant solution after centrifugation was extracted sequentially with ether and di-(2-ethylhexyl)phosphoric acid in chloroform. The aqueous solution was then subjected to reversed phase liquid chromatography on a 25 cm ODS column with a mobile phase consisting of methanol: 0.03M sodium phosphate buffer containing 62mM of methanesulphonic acid and 0.1mM EDTA (3:97). An electrochemical detector fitted with a glassy carbon electrode operated at a potential of 0.75V was used for detection. After two weeks of treatment the mean content of 5SCD in control skin was 346±28(S.E.M.) ng/g of tissue (wet weight) and for HQ treated skin the mean content was 382±22 ng/g. This difference is not statistically significant ($p>0.05$). However, after four weeks of treatment the mean content of control skin was 290±18 ng/g and for HQ treated skin the mean content was 197±15 ng/g. This difference is significant ($p<0.01$). Thus, HQ treatment was found to decrease the content of 5SCD in epilated black guinea pig skin after four weeks of treatment.

ANALYSIS OF MULTIFREQUENCY ESR SPECTRUM OF DOPA-MELANIN
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The electron spin resonance (ESR) spectrum of an aqueous suspension of synthetic DOPA-melanin at pH 1.0-12.0 is a superposition of anisotropic lines arising from different protonation states of radical species [1]. At Q-band (35 GHz) a melanin ESR spectrum can be simulated assuming a Gaussian lineshape function for each of the composite lines. However, at lower frequencies (9.5 and 2.4 GHz) this assumption leads to a poor fit. To solve this inconsistency, possible mechanisms of the melanin line broadening and their contributions to the overall lineshape function were considered. The first mechanism, arising from relaxation processes, gives rise to the Lorentzian lineshape function of the spin packet. A second mechanism, arising from an unresolved hyperfine interaction with protons, and a third, arising from a distribution of the g-tensor principal values due to the heterogeneity of the polymer, give rise to the Gaussian lineshape function. Thus, the resulting lineshape function is Gaussian-Lorentzian convolution. Using this lineshape function, very satisfactory fits between simulated and experimental ESR spectra for melanin at a wide range of pH values are obtained. Parameters giving the best fits contain information about the residual linewidths of spin packets, the heterogeneity of the radical species and their interaction with surrounding protons.

[1] Pasenkiewicz-Gierula, M. and Sealy, R. C. (1986) BBA (submitted).

INTERACTION OF OXYGEN WITH SEMIQUINONE PRECURSORS OF MELANINS. P. Hintz and B. Kalyanaraman, Medical College of Wisconsin, Milwaukee, USA; E.J. Land, Paterson Labs., Manchester, U.K.; B. Pilas and T. Sarna, Jagiellonian Univ., Krakow, Poland; T.G. Truscott, Paisley College, Scotland, U.K.

Certain semiquinones (SQ) are known for their high reactivity with molecular oxygen. The superoxide anion ($O_2^{\cdot-}$) produced by this interaction is regarded as a potential cytotoxic species in many biological systems and it has been suggested that SQ may arise during melanogenesis and hence lead to $O_2^{\cdot-}$ and H_2O_2 production. In an attempt to confirm such processes we have studied oxygen consumption using the horse radish peroxidase (HRP)/H_2O_2 system to produce the SQ (confirmed by esr) of DL, β-dopa (dopa), 1,2,4-benzenetriol (BT), 6-hydroxydopa= 2,4,5-trihydroxyphenylalanine (TOPA), and 5-S cysteinyldopa (5-SCD). A substantial increase in oxygen consumption is observed in the presence of TOPA and BT but a much smaller increase with the other SQ studied. Pulse radiolysis allowed direct determination of the rates of reaction of the oxygen with the SQ (generated by oxidation via the azide radical, $N_3^{\cdot}$). For TOPA and BT semiquinones we obtained a second-order reaction rate constant of $2\text{-}3\times10^6$ $M^{-1}s^{-1}$ whereas for the other SQ the corresponding rate constants were $< 10^5$ $M^{-1}s^{-1}$. Thus, while the production of H_2O_2 is established during melanogenesis our results imply that dopa SQ is not the major source of this species.

REEXAMINATION OF THE STRUCTURE OF EUMELANIN. Shosuke Ito. School of Hygiene, Fujita-Gakuen Health University, Toyoake, Aichi, Japan.

The generally accepted concept that the black melanin eumelanin is made mostly from 5,6-dihydroxyindole but not from 5,6-dihydroxyindole-2-carboxylic acid (DHIC) was reexamined by comparison of synthetic and natural eumelanins. The analytical methods used were elemental analysis and determination of carboxyl group by acid treatment (hydrolysis) to yield CO_2 and by permanganate oxidation to yield pyrrole-2,3,5-tricarboxylic acid. It was found that DHIC-derived monomer units comprise only ca. 10% of enzymically prepared dopa-melanins but as much as a half of natural eumelanins present in melanosomes from sepia and B16 melanoma and C57 black mouse hair. Thus, it appears that DHIC could play a more important role in the biosynthesis of eumelanins than previously believed. The results also show that dopa-melanins prepared at higher pH retain higher percentages of the carboxyl group of dopa and contain higher percentages of pyrrole units, and that melanins are decomposed to significant extents on acid treatment, the method commonly used to isolate melanins from natural sources.

THE RELATIONSHIP BETWEEN TWO MELANOMA CELL SUB-POPULATIONS WITH DIFFERENT ELECTROPHORETIC MOBILITIES

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It has previously been reported that the population of pigmented Bomirski hamster melanoma (BHM) cells consists of two types of cell revealing different electrophoretic mobiliy (EPM): of a "slow-moving" and "fast-moving" subpopulation. This is a reflection of different cell surface charge densities in these two sub-populations. The question arose as to the relationship between these two types of cell. To answer this question the electrophoretic profiles of cell populations derived from tumors and in vitro cultures were studied using a conventional, vertically positioned Abramson's cell. It was found that the percentage of "fast-moving" cells depends on the age and size of tumors. The larger the tumor the greater the proportion of "fast-moving" cells. In vitro cultivation of pigmented BHM cells causes the percentage of "fast-moving" cells first to decline rapidly and then to increase to approximately 60%. The electrophoretic profiles of non-pigmented BHM cells which start producing melanin under in vitro conditions are identical to those of melanotic cells. It is concluded that the "fast-moving" subpopulation originates from the "slow-moving" one as a result of their aging and differentiation.

SYNTHESIS OF 5-S-L-CYSTEINYL-GLYCINE-L-DOPA FROM GLUTATHIONE-L-DOPA WITH GLUTAMYLTRANSPEPTIDASE

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One postulated in vivo pathway for synthesis of 5-S-L-cysteinyl-L-dopa (CD) is that glutathione (GSH) by nucleophil addition to dopaquinone forms glutathione-L-dopa (GD). GD is then by γ-glutamyltranspeptidase (GT) converted to 5-S-L-cysteinyl-glycine-L-dopa (CGD) and further with a dipeptidase to CD. This pathway was followed to synthesize CGD for use as dipeptidase substrate.

Methods: GD was synthesized from GSH and L-dopa with tyrosinase and purified on an AG 50W-X4 column. With GT from kidney GD was converted to CGD which also was purified on an AG 50W-X4 column and by preparative HPLC.

Results: The yield of GD varied between 36 and 55%. GT converted the GD rapidly into CGD but also into CD. After purification of the GT preparation most dipeptidase was eliminated, and with such a preparation the yield of CGD was about 50%. On purification of CGD on AG 50W-X4 and preparative HPLC the yield was about 30%. The whole procedure gave a yield of about 10% and resulted in a CGD preparation free from other L-dopa-thioether compounds as judged from analytical HPLC.

Conclusion: The method for synthesis and purification gave a preparation of CGD which was free from CD and could be used as substrate in the detection of a dipeptidase which gives CD as product.

PHOTOCHEMISTRY OF MELANIN PRECURSORS: DOPA AND CYSTEINYLDOPAS. Walter H. Koch*, Ambler Thompson[1] Miles R. Chedekel*, Edward J. Land** and T. George Truscott[1] *The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD. U.S.A., **Patterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, U.K. and [1]Dept. of Chemistry, Paisley College, Paisley, U.K

It is our hypothesis that the photochemical mechanisms operative in UV-induced skin cancers and aging ,involve the photochemistry of cysteinylcatechols, natural metabolites of active melanocytes. Thus we have investigated the photochemistry of dopa, and cysteinyldopas 5SCD, and 2,5SCD using laser flash photolysis (LFP), ESR spin trapping, and photoproduct analysis. LFP of dopa provided evidence for formation of dopasemiquinone via two primary photochemical mechanisms: photoionization (giving e^-_{aq}) and photohomolysis (giving H). Confirmatory evidence for these radical pathways was provided by spin trapping with DMPO and ESR spectroscopy. In contrast, LFP of 5SCD and 2,5SCD results in lower photoionization quantum yields (1.2 and <0.5, respectively) and the production of initial transient species whose absorption spectra were markedly different from their semiquinone absorption spectra previously determined pulse radiolytically. Using ESR spin trapping with DMPO, we found evidence for the production of a carbon-centered radical species during 5SCD photolysis. Further characterization by use of a nitroso spin trap, MNP, demonstrated that homolytic cleavage of the -S-CH_2- bond of the 5SCD cysteinyl side chain is a significant photochemical pathway. Analyses of the photoproducts formed are in progress and the results of these studies will be discussed.

REACTIVITY OF EU- AND PHAEOMELANINS WITH SUPEROXIDE
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1-Medical College of Wisconsin, Natl. Biomedical ESR Center, Milwaukee, U.S.A.

We have previously shown that melanin pigments can act as a pseudo-dismutase. In the present studies various eu- and phaeomelanins were compared with respect to their reactivity with superoxide radicals.

The molecular basis of the interaction of superoxide anion with different melanins was studied.

The differences between eu- and phaeomelanins are discussed in terms of their possible photoprotective (phototoxic) role in the skin.

VISCOSITY AND RIGIDITY IN COLLOIDAL SOLUTION OF MELANINS: Ryusuke Kono and Shinji Ota: Dept. of Applied Physics, The National Defense Acad., Yokosuka, Japan

Shear wave measurement was conducted on aqueous solution of synthetic melanins in the frequency range from 5.3 to 320 MHz. It was found that shear spectrum in Dopa melanin for a weight fraction Cw= 0.1 is quite similar to that in diethylamine (DEA) melanin for Cw=0.3. This similarity may indicates that the both melanins consist of rod-like molecules in an amorphous phase and DEA behaves itself like a plasticizer. Concentration dependency of viscoisty in DEA melanin ranging from Cw=0.1 to 0.3 follows in Kirkwood-Auer-Doi thory based on Brownian motion of stiff-chain molecule.

The mechanical property changes slowly for a long time scale. So called syneresis was analyzed in terms of limiting shear modulus, aging of which stems from the more packed rearrangement of planner group.

LIGHT SCATTERING CALCULATIONS FOR MELANIN PIGMENTS FROM THE RAYLEIGH TO THE MIE REGIME.
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Using the wavelength dependent optical "constants" $n(\lambda)$ and $k(\lambda)$, determined in a separate study, we have calculated the extinction, scattering and absorption cross-sections over the visible spectrum for individual granules of melanin assumed to be spherical and optically homogeneous. The calculations were carried out for particle radii r from 0.001 μm to 10 μm. In the Rayleigh regime ($r < .025$ μm), absorption is found to dominate scattering, a result which does not support the Wolbarsht model.

For monodispersive suspensions of larger granules ($r \geq 0.1$ μm) in H_2O, KBr and keratin we find absorption and scattering contribute nearly equally to the optical density, with the wavelength dependence coming mainly from $k(\lambda)$ but modifed by Mie scattering. These modifications depend strongly on particle size. Comparison with the optical density data of Blois on sepia melanin in KBr, and Matsumoto, Toda, and Fitzpatrick on melanosomes in mouse hair follicles, shows that the observed wavelength dependence is reproduced by the present calculations.

OPTICAL CONSTANTS OF SOLID MELANINS DETERMINED FROM REFLECTION MEASUREMENTS IN THE VISIBLE SPECTRUM.
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By using a combination of external (pseudo-Brewster angle) and internal (Total Internal Reflection) specular reflection measurements, we have been able to measure the real (n) and imaginary (k) parts of the complex index of refraction N, $N(\lambda) = n(\lambda) - ik(\lambda)$, for wavelengths λ from .35 μ to .8 μ.

For the sample of sepia melanin MFA, we find n = 1.655 $\pm$ .008 and k = .12 $\pm$ .07 at 632.8 nm. The imaginary "constant" k, which is related to the absorption coefficient $\alpha(\lambda) = [4\pi/\lambda]\, k(\lambda)$, is found to be strongly dependent on wavelength, decreasing rapidly toward longer wavelengths as $k(\lambda) = a(1-b\lambda)^2$ where a and b are material constants independent of wavelength with values close to unity for optical wavelength measured in microns (μm).

Comparison of these results with measurements made on transmission through thin films will be given. A discussion will be presented of the dependence of the optical constant parameters a and b on physico-chemical variables such as moisture content, chemical composition (including other types of melanin), density and temperature along with a possible theoretical interpretation of a and b based on an amorphous semiconductor model. Sample preparation methods which were a key element in obtaining the specularly reflecting surfaces required for these measurements will also be described.

THE FIRST ELECTRON SPIN RESONANCE (ESR) IMAGE OF A MURINE MELANOTIC TUMOR
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In contrast to huge advances in NMR imaging, tomography based on the ESR techniques is still at an early stage of development. The ESR images so far reported were mainly made at the X-band using solid-state samples (Ohno, J.Magn.Res.49,56,1982). Only recently Fujii and Berliner were able to obtain the ESR images of aqueous phantoms and plant specimens (Magn. Res. Med., 2, 275, 1985).
The present paper describes the first successful attempt at imaging an S91 Cloudman melanoma growing on the tail of a DBA mouse. The size of the tumor amounted to 8 mm in diameter. Visualization of the internal structure of the tumor was accomplished through the intravenous administration of a spin label (CTPO). The ESR spectrometer operated at 1.55 GHz, utilizing a flat loop coil and a magnetic field gradient of 1.1 Gauss/mm. The results were digitized in a Varian E-935 data system.
The central necrotic area with poor vascularization and low level of O_2 could be clearly distinguished in this ESR image from the zone of active tumor growth. Information provided by ESR imaging about the spatial distribution of O_2 and paramagnetic centres, including in particular NMR contrast agents, within the organism, is of great value for radiotherapy and basic research on NMR diagnostics.

COMPARTMENTALIZATION OF TYROSINASE AND DCF ACTIVITIES IN EUMELANIC AND PHEOMELANIC CELLS. Dr. M. Lynn Lamoreux, Biology, Texas A & M University, College Station, TX 77843. The agouti locus of the laboratory mouse determines whether the melanocytes will produce pheomelanin or eumelanin. The agouti-locus control is exerted via the tissues that surround the melanocyte, rather than autonomously within the melanocyte. This control apparently involves modulation of cyclic-AMP activity within the melanocyte, and results in changes in the catalytic activities of at least two enzymes, tyrosinase and dopachrome conversion factor (DCOR). DCOR activity is absent from whole-cell extracts made using tissues that are producing pheomelanin, and the dopa oxidase activity of tyrosinase is reduced in these extracts compared with those made using eumelanic tissues. Extracts are equilibrated on the basis of the tyrosine hydroxylase activity of tyrosinase. I have now measured the catalytic activities of the microsomal, melanosomal and soluble fractions of pigmented tissues. Dopa oxidase activity is similarly high in the microsomal fractions, but is much reduced in the melanosomal fraction of pheomelanic tissues. Compartmentalization of dopachrome conversion factor activity differs from that of dopa oxidase activity.

DETECTABILITY OF MELANIN CONTAINING TISSUES BY NMR IMAGING TECHNIQUE
S.J. Lukiewicz*, S.G. Lukiewicz, Medical College of Wisconsin and National Foundation for Cancer Research, Milwaukee, * on leave from the Jagiellonian University, Krakow, Poland, F. Wehrli, N. Grigsby, General Electric Medical Systems, Milwaukee, U.S.A.

Separate in vivo ESR and NMR studies demonstrated that melanin may behave as a weak NMR contrast agent and can bind several types of NMR contrast enhancing substances (Lukiewicz, Wehrli, Grigsby, Lukiewicz, Intern. Symp. on Radiopharmacol., Freiburg, FRG, p. 45, 1983). Contrast enhancing properties of melanins present in animal tissues, and in particular in melanotic tumors, can be effectively increased in this way (Lukiewicz, Wojcik, Marczynska, Persson, Olsson, V Annual Meeting Soc. Magn. Res. in Medicine, Montreal, 1986, submitted).
The NMR images presented in this paper show that the melanin content of the cell can in fact improve the detectability of melanotic tissues. This was clearly revealed by comparing the NMR images of the amelanotic and melanotic murine melanoma, obtained using a 1.5 Tesla General Electric NMR scanner, and the inversion recovery or spin echo pulse sequences. The increase in brightness of the pigmented melanoma image was too small, however, to be of practical value for NMR diagnostics. The NMR images of the melanoma bearing mice treated with the tumor-seeking melanoma-specific NMR contrast agents indicate that the prospects of early detection of small melanoma metastases may be quite good in this case.

IN VIVO ESR MEASUREMENTS OF OXYGEN CONCENTRATION IN MURINE MELANOMAS GROWING IN SITU

S. Lukiewicz, A. Sochanik*, W. Subczynski, J. Hyde*
*Medical College of Wisconsin, Milwaukee, Wisconsin
Jagiellonian University, 31-120 Krakow, Poland

The present paper demonstrates that the newly developed in vivo ESR technique (S.J. Lukiewicz and S.G. Lukiewicz, Magnetic Resonance in Medicine, 1, 297, 1984) can be combined with spin label oxymetry and may offer a chance of monitoring the level of oxygenation in large biological objects, including in situ growing tumors.
The in vivo ESR determinations of O_2^- concentration were carried out at 1.3 GHz using a Varian E-9 spectrometer or at 3.2 GHz using a Radiopan SE/X machine. The model of B16 melanomas growing on the tails of C57Bl mice proved to be suitable for this kind of study. Several nitroxides were chosen. They were given intravenously and the amplitude of their ESR signals was followed for several hours. The rates of reduction and reoxydation of the spin labels and their biological half lives could be estimated from the thus obtained pharmacokinetic curves.
It was found that the rate of reduction of a given spin label under in vivo conditions depends on the level of oxygen in the tumor. Therefore, comparison of the above pharmacokinetic parameters provided quantitative information about the O_2 content of tumors. It is concluded that the in vivo ESR results confirm the view, based on theory, that the pigmented tumors actually tend to develop a state of deep oxygen deficiency.

SYNTHESIS AND ANALYSIS OF PHEOMELANIN DEGRADATION PRODUCTS. 3.

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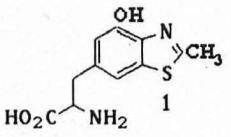
Hydroiodic acid degradation of pheomelanin affords a unique set of amino acids. Detection and quantitation of several of these degradation products has been used as evidence for the presence of pheomelanin in melanin containing tissues. Only one of the eight major degradation products is commercially available, and during the past six years we have published the synthese.s of an additional three of these amino acids. In the present work we describe the syntheses of three of the remaining four degradation products (1-3) and an HPLC protocol for separation and quantification of the seven available HI degradation products.



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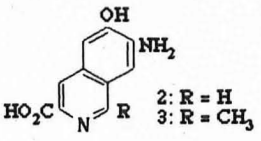

2: R = H
3: R = CH_3

MELANINS AS NATURAL, ENDOGENOUS NMR CONTRAST AGENTS AND BINDING SITES FOR EXOGENOUS CONTRAST AGENTS

S. Lukiewicz, K. Wojcik, E. Markowska
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The NMR contrast agents used in NMR imaging are, as a rule, paramagnetic. The paper indicates that melanins also reveal a paramagnetism strong enough to affect the proton relaxivity. This has been shown by in vitro and in vivo ESR and NMR techniques. Radiopan SE/X ESR and Praxis II pulsed NMR spectrometers were used to measure the appropriate parameters. The T1 value for water (3400 ms at 10.7 MHz) was found to fall to 2671 ms after the addition of melanin (70 mg/ml). The T1 of an in situ growing B16 melanoma amounted to about 600 ms. This means that one can hardly expect any strong contrast enhancement to result from the natural paramagnetism of melanins. Advantage can be taken of the fact, however, that melanins can bind many types of metal ions and free radicals. Hence, various NMR contrast agents tend to accumulate in melanotic cells, e.g. the T1 of B16 melanoma was shown to decrease from 600 to 150 ms after several injections of 5.10^{-3} M GdDTPA. In vitro incubation of melanin with this compound drastically reduces the T1 value of melanin (from 2671 to less than ms). The findings indicate that weak contrast enhanceing properties of natural melanins can be very effectively potentiated by suitable contrast agents, especially if they are capable of penetrating the cell membrane of melanoma cells and binding to melanins.

ELECTROCHEMICAL PROPERTIES OF NATURAL AND SYNTHETIC MELANINS

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The standard redox potential ($E_0^{\cdot}$) is among the basic physico-chemical parameters which determine the donor-acceptor properties of a compound. It has not so far, however, been estimated, for melanins.
A new method has recently been developed by one of the authors (Z.M.) which makes it possible to determine E_0 on the grounds of acid-base titrations under simultaneous control of the redox potential (E_n) and the intensity of ESR absorption (A_{ESR}) of melanins. The standard redox potential E_0 can be calculated by extrapolation from the dependences between E_n and pH, A_{ESR} and pH, and E_n and A_{ESR}. This paper briefly describes the results of such a study. The acid-base titrations were performed using platinium (Pt) and saturated calomel electrodes (SCE). A Varian E-3 ESR spectrometer was used to measure A_{ESR}. For the redox titrations 0.1 N Ce $(SO_4)_2$, 0.05 M $KMnO_4$, and 0.1 N chloramine were chosen. The acid-base and redox titrations were found to give a similar value for E_0. In both cases the estimated value of the standard redox potential E_0 amounted to 0.7 - 0.8 V (NHE). The synthetic DOPA-melanin and the natural AB melanin from the bovine eye did not differ substantially in this regard.

PROPERTIES OF MELANINS ISOLATED FROM HUMAN BLUE AND BROWN EYES. I.A. Menon, P.K. Basu, H.F. Haberman, S. Persad, M. Avaria, C.C. Felix and B. Kalyanaraman. Departments of Medicine and Ophthalmology, University of Toronto, Canada and National Biomedical ESR Center, Medical College of Wisconsin, Milwaukee, Wisconsin, U.S.A.

Investigations were carried out to determine whether the melanin present in the blue and brown eyes (BlEM and BrEM respectively) were eumelanin, the melanin present in black hair and dark skin; or pheomelanin, the melanin present in red hair and the skin of people with red hair. Our results show that UV-visible irradiation of BlEM or BrEM did not produce any superoxide. Irradiation of ^{51}Cr-labeled Ehrlich ascites carcinoma cells in the presence of BlEM or BrEM did not produce significant cell lysis. The ESR signals of these melanins were very similar to those of eumelanin. Comparison of these findings with the previous results indicate that BlEM and BrEM are essentially eumelanin. The ESR signals further suggest that with regard to both blue and brown eye melanins, the iris, ciliary body, choroid and retinal pigment epithelium did not differ. The binding of three drugs, viz. 8-methoxypsoralen, imipramine and epinephrine to BlEM and BrEM were also studied. These drugs formed complexes with both BlEM and BrEM. However, there were differences in the binding sites and association constants for the binding of these drugs to the two melanins. (Supported by MRC and NIH).

EVIDENCE FOR A FREE-RADICAL MEDIATED OXIDATION OF MINOCYCLINE

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Minocycline, 7-demethylamino-6-deoxy-6-demethyltetracycline, a semisynthetic derivative of tetracycline is an antibiotic used for the treatment of Acne Vulgaris. Thyroid and skin pigmentation have been reported in patients on chronic minocycline therapy, although no direct toxic effects of minocycline associated pigmentation have been reported. Our studies indicate that this pigmentation is probably a polymer derived from the oxidation of minocycline, and thus we have studied the early steps in the oxidation of minocycline.

Oxidation of minocycline under slightly alkaline conditions produces a transient free-radical with hyperfine splittings characteristic of a phenoxy radical. A different radical is observed at high pH and metal-complexed radicals are observed with calcium and strontium. The radical is formed by reverse dismutation of a quinoid species and its reduced form. The reduced form of the quinoid species was isolated and found to be 7-hydroxy-6-deoxy-6-demethyltetracycline. The initial oxidation of minocycline is a two-electron oxidation with no free radical production. Hydrolysis of the two-electron oxidation product gives the parent quinoid of the free radical.

A RESTRICTION AND BUOYANT DENSITY STUDY OF 5,6-DIHYDROXYINDOLE MODIFIED DNA.
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The restriction patterns of λ phage and E.coli DNAs by several restriction endonucleases (Alu I,Aha III,Bal I,EcoRi,Hha I,Hind III,Hpa II,Pvu II,Sma I) were compared to those obtained using the same DNAs treated with 5,6-dihydroxyindole (DHI) and/or U.V. irradiation. Controls and treated DNAs were also compared by CsCl buoyant density centrifugation. Previously it was demonstrated DNA modification by L-DOPA oxidation cytotoxic products (Miranda et al.Mol.Gen.Genet.1984-193:400-405) while in the present study the action of a particular cytotoxic intermediate of melanin synthesis, DHI (Pawelek and Lerner-Nature.1978-276:627-628),has been investigated and the conclusions were: a)DHI modifies the DNA electrophoretic mobility and buoyant density profile;b)UV irradiation enhances DHI or DHI autooxidation products binding to DNA as previously shown for tryptophan;c)DHI binding to DNA is not sequence specific as evidenced by restriction.

EFFECT OF METAL IONS ON THE CONVERSION OF DOPACHROME TO MELANIN.

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In a recent study on the effect of zinc ions on the rearrangement of dopachrome,a major control point in the biosynthesis of eumelanins,we have found that the reaction leads mainly to 5,6-dihydroxyindole-2-carboxylic acid (DHICA) rather than to 5,6-dihydroxyindole (DHI),as previously believed.We report now that other divalent cations,e.g.Cu^{2+},Co^{2+},are much more effective in catalysing the conversion of dopachrome to DHICA. As a rule,the extent of the observed effect is dependent on the concentration of the metal added and is suppressed by EDTA.When considered in the light of the known metal accumulation in pigmented tissues, this finding suggests that DHICA may be an important, ultimate precursor of melanins. Support to this view has been obtained by $KMnO_4$ degradation of natural and biosynthetic melanins prepared from dopa in the presence and in the absence of various metal ions.Quantitative analysis of pyrroletricarboxylic acid (PTCA), a typical oxidation product of DHICA, showed that in the presence of metal ions incorporation of DHICA in the polymer occurs to a considerably higher extent with respect to control melanin prepared in the absence of metals. Taken together,these results provide evidence for the critical role played by metal ions in the regulation and control of melanin biosynthesis.

INTERACTION OF DOPA-MELANIN WITH Fe^{2+} AND Fe^{3+} AT LOW pH. M. Pasenkiewicz-Gierula and Wojciech Froncisz, Jagiellonian University Krakow, Poland; National Biomedical ESR Center, Medical College of Wisconsin, Milwaukee, WI 53226 U.S.A.

The interaction between iron and melanins is of a great interest due to redox and ion-exchange properties of melanins as well as the biological importance of iron. The synthetic DOPA-melanin was chosen as a well-defined model of natural eumelanin. The reactions between melanin and ferric and ferrous ions were investigated in acidic media (∿ pH 2.0) under aerobic and anaerobic conditions. Using spectrophotometric and electron spin resonance (ESR) techniques it was shown that both oxidation and reduction reactions as well as binding occur in melanin-iron system. Typically, the process of chelation of iron to melanin reaches equilibrium within a few minutes (i.e. less than 5 min.), whereas a steady state for redox reaction is reached after approximately 24 h. The amount of ferrous ions bound to melanin was less than the amount of ferric ions. Based on ESR and Mossbauer spectroscopy results it seems likely that initially formed melanin-Fe(II) complex undergoes oxidation with time to form melanin-Fe(III) complex. The amount of iron oxidized or reduced by melanin depends on the redox potential and capacity of melanin.

SPECROPHOTOMETRIC AND EPR SPIN TRAPPING STUDY OF THE INTERACTION OF CHELATED IRON IONS WITH MELANINS
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Electron Paramagnetic Resonance (EPR) was used for detection of the spin adduct in a Fenton system containing ferrous and ferric ions (chelated with EDTA or DTPA), H_2O_2, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and dopa-melanin or cysteinyldopa-melanins. The electron transfer processes between the melanin and Fe[II]/Fe[III] were monitored by spectrophotometric measurements of either oxidised melanin or reduced iron ions. Melanins show some inhibitory effect on the OH-radical formation in a typical Fenton system as judged by EPR and spectrophotometric experiments. A dramatic effect of melanin on hydroxyl radical generation was observed in samples containing H_2O_2, chelated ferric ions and DMPO with the rate of DMPO-OH adduct formation being dependent on the concentration of melanin and Fe[III] and the type of the pigment. Oxygen had a little effect on this "melanin driven" Fenton reaction; the reduction of ferric ions by melanin occured even in oxygen saturated samples. Thus, in certain systems, melanin can be considered as an efficient promoter of the Fenton reaction.

PHOTOSENSITISATION OF MELANINS COVALENTLY BOUND TO DYES by B. Pilas and T. Sarna, Jagiellonian Univ., Krakow, Poland and C.N. Knox and T.G. Truscott, Paisley College, Scotland, UK. We have previously studied the interaction of porphyrin dyes with synthetic melanins and shown that the strong binding with cationic dyes leads to complete quenching of all detectable photochemical properties while there is no effect on the anionic dyes. We now report studies of melanins when covalently bound to a dye. Laser flash photolysis (lfp) and O_2 consumption (oc) have been used to study erythrosin (ery) covalently bound to a series of melanins: dopa melanin (DM), solubilised squid melanin (SSM) and 5-S cysteinyl-dopa melanin (5S-CP). The oc with DM covalently bound to ery (DM-ery) showed marked quenching due to azide ion. Adding unbound DM to the DM-ery had no effect on the rate of oc. This implies that singlet oxygen produced by the dye when covalently bound only leads to oc by reaction with the DM in close proximity to the dye. Lfp data gave fast oxygen quenching of the triplet of (DM-ery) $\sim 10^9\ M^{-1}s^{-1}$ but with only slight azide quenching of the triplet $\sim 10^5\ M^{-1}s^{-1}$. This result confirms that, in the oc studies, the effect of azide is not to quench the triplet but rather the singlet oxygen. Quantum yields of triplet production (Φ_T), were 0.026, 0.047 and 0.034 for DM, SSM and 5S-CP covalently bound to ery respectively while for ery unbound we obtained a Φ_T of 0.25. Reduction in fluorescence yield was also noted for this dye on melanin binding. Our results show that selective dye sensitisation of melanin can be achieved by covalent binding the dyes.

FREE RADICAL PROPERTIES OF NATIVE AND SOLUBILIZED EU-AND PHEO-MELANINS
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AB-melanin isolated from bovine eyes, intact and chemically modified sepia ink melanin and synthetic eumelanins derived from dopa and dihydroxyindole were compared with pheomelanins (isolated from red hair and synthesized from cysteinyldopas and cystein-dopa mixtures) by means of electron spin resonance spectroscopy (ESR). The integrated intensity, lineshape and g-factor of the melanin ESR signals were studied as a function of pH, zinc-ion-doping and light illumination. Our results reveal striking dissimilarity in the ability of various melanins to respond to the action of typical inducers of melanin radical formation. Chemical alteration of the polymer, induced by the solubilization procedure, is probably responsible for the reduction in number of the melanin active centers. It can also be speculated that structural differences between the pheo- and eu-melanins significantly affect comproportionation equilibria in both polymers.

INFLUENCES OF LIGHT (Blt) AND SILVER (si) GENES ON EUMELANIC AND PHEOMELANIC COAT COLOR IN THE MOUSE.
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Melanocytes disappear prematurely from the anagen hair bulbs of light (a/a; Blt/Blt) and silver (a/a; B/b; si/si) mice. As a result, most hairs are pigmented at their tips and white at their bases. The extent of basal depigmentation is increased in light-silver (a/a; Blt/Blt; si/si) mice. Large clumps of eumelanin (whole or substantial fragments of melanocytes) are found in hairs of light, silver and light-silver mice. Atypical densely pigmented melanocytes are evident on day 5 of a hair cycle in light-silver mice, a day or more before they appear in light or silver mice. The Blt and si genes may act synergistically to bring about abnormal melanogenesis followed by premature death and dislodgment of follicular melanocytes.

Yellow (pheomelanic) (Ay/a; B/b; si/si) silver mice show clumping and basal dilution of pigment within their hairs. The pigment loss is variable but markedly less than that found in corresponding eumelanic silver mice. Since traces of eumelanin and silvering diminish in the pelage of yellow-silver mice as they age, it is possible that both the Blt and si genes adversely influence melanocytes which at least temporarily synthesize some eumelanin.

REDOX PROPERTIES OF MELANINS. T. Sarna+, E.J. Land+ B. Pilas+ and T.G. Truscott*. +Jagiellonian Univ., Krakow, Poland, † Paterson Labs., Manchester, UK., * Paisley College, Scotland, UK. The protective role of melanins may well be related to the reducing properties of certain sites in the melanin molecule. We have studied the reaction of the bipyridylium quaternary salt: diquat (DQ^{2+}), paraquat (PQ^{2+}) and triquat (TQ^{2+}) radicals (reduction potential -350, -450 and -550 mv for $DQ^{\cdot+}$, $PQ^{\cdot+}$ and $TQ^{\cdot+}$, respectively) produced by pulse radiolysis in the presence of various synthetic melanins. For $DQ^{\cdot+}$ and $PQ^{\cdot+}$ only little reaction (electron transfer) with autooxidative Dopa-melanin (DM) is obtained implying few reactive sites of DM to this reducing reagent. However, 5-S cysteinyldopa-melanin (5SCP), 2,5-SS'dicysteinyldopa-melanin (2,5-SSCP) polymers of dopa with cysteine and solubilised squid melanin (SSM) all showed much more marked second-order reaction with $DQ^{\cdot+}$ with bleaching of the melanins being detected at 605 nm. Similar behaviour was found with $PQ^{\cdot+}$, with 2,5-SSCP again showing more reaction and melanin bleaching. For the more reducing $TQ^{\cdot+}$ a marked reaction with DM was detected implying that many more sites in DM are susceptible to attack by this strong reducer (> 10 × that with $DQ^{\cdot+}$ and $PQ^{\cdot+}$). Thus for DM the major reactive sites have a 1 electron reduction potential between -450 and -550 mv. Also, repetitive pulsing showed for all melanins studied, > 1 type of site with respect to redox properties. For all phaeomelanins as well as SSM it seems that the major reduction potential is more positive than -350 mv.

THE REACTIVITY OF MELANINS WITH OXYGEN RADICALS AND OTHER POTENTIALLY CYTOTOXIC SPECIES GENERATED BY IONIZING RADIATION AND UV-VIS ILLUMINATION
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Postulated protection of melanins against cellular damage caused by ionizing radiation and/or ultraviolet-visible light illumination may involve scavenging of water radiolysis products, certain organic free radicals and electronically excited molecules. The effectiveness to scavenge free radicals, or any other reactive species, depends on both - the local concentration of the scavenger and the biomolecular rates of specific reactions. Highly heterogeneous distribution of melanin granules within pigmented cells is an important factor to be considered when speculating about melanin protective role. Critical review of the reactivity of eu- and pheo-melanins with hydroxyl radicals, superoxide anion, hydrated electron, hydrogen peroxide, singlet oxygen and some singlet and triplet exited dyes is presented. The techniques used to study these processes (electron spin resonance, ESR-oxymetry, absorption and fluorescence spectroscopy, laser flash photolysis and pulse radiolysis) and the experimental conditions that affect the rate constants of such interactions are briefly described.

ESTABLISHMENT OF A MOUSE MELANOCYTE CLONE WHICH SYNTHESIZES BOTH EUMELANIN AND PHEOMELANIN, AND ITS RESPONSE TO L-DOPA.

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Epidermal cells from 3.5 day-old C57BL/6J mice were cultured in the medium (MEM) containing PMA (16nM) and cholera toxin (10nM). In these conditions, melanocytes proliferated selectively. We cloned some of cell lines from these cell populations. One of the clone, TM 10, has normal chromosomes and possesses tyrosinase activity. The quantities of eumelanin and pheomelanin were chemically determined by their degradation products, PTCA and AHP. The cells were shown to contain equal amount of eumelanin and pheomelanin. In this cell line, both eumelanosomes and pheomelanosomes were observed with electron microscopy. Mosaic-type melanosomes were also observed: fine structures of the eumelanosomal type and pheomelanosomal type were seen in a single melanosome.

When TM 10 cells were cultured in the medium containing DOPA ($2X10^{-4}M$), a drastic increase in the content of pheomelanin was observed. Pheomelanosomes in each cell were shown to increase in number with electron microscopy. It was suggested that average tyrosinase activity in each melanosome was decreased.

CHELATION OF 5,6-DIHYDROXYINDOLE WITH DIVALENT METALS.

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The reactivity of 5,6-dihydroxyindole (DHI) *in-vivo* is very much a function of its interaction with metal-ions. Although much importance has been attached to this phenomenon, little is known of the nature of the reactive species formed between DHI and specific metal ions. The extent of complexation was determined by measuring the Δ O.D. at 350 nm of the UV-visible spectrum of DHI in anaerobic aqueous medium upon the serial addition of either Cu^{+2}, Ni^{+2}, Co^{+2}, or Zn^{+2}. From the non-linear plots of Δ O.D. *versus* [metal-ion] at varied pH, the complexation constants were calculated. Under anaerobic conditions, copper(II) ion reacts immediately with DHI to give a melanochrome species (λ_{max} ~600 nm) but Ni^{+2} reacts very slowly; Co^{+2} and Zn^{+2} do not form the melanochrome. The subsequent addition of air to these anaerobic solutions results in more rapid formation of the melanochromes followed by their disappearance. A comparison of the relative rates of formation and loss of the melanochrome shows that both processes are pH dependent and accelerated by metal ions in the order Cu^{+2} >> Ni^{+2} > Co^{+2} > Zn^{+2}. Each metal ion catalyzes the production of different melanochrome species as shown by the different λ_{max} values and by HPLC of their reaction mixtures after reductive acetylation.

Melanochrome formation apparently involves one electron oxidation by the metal ion as seen by the Cu^{+2} and Ni^{+2} anaerobically-initiated reactions.

LONG LASTING DIELECTRIC POLARIZATION OF PHEOMELANIN AND ALLOMELANIN J. Slawinski, W. Osak & K. Tkacz, Institute of Physics, Pedagogical University, 30084 Cracow, Poland.

The possibility that submolecular and molecular electronic properties of melanins may be relevant to their physiological functions is more plausible when it is remembered that melanin in vivo, e.g. in melanosome membranes are subjected to electric field gradients of 10^7 Vm^{-1} or more. This consideration prompt us to measure dielectric and electronic properties of natural and model synthetic melanins under conditions modeling physiological ones. Natural pheomelanin obtained from red human hairs and synthetic allomelanin prepared by the oxidative polymerization of catechol according to standard procedures were used. EPR, IR, UV and visible (optical) spectra and dielectric constant and specific resistance were determined to characterize their properties. The measurements of thermic depolarization were performed with air-dry samples. A sample in the form of a thin disc deposited on a Pt-electrode was polarized in the electric field of 30-3000 Vcm^{-1} for the polarization time 10 s - 2 hr. The results of the measurements show that the depolarization process is the long-lasting one, in the order of a few days. It suggests the presence of an electret effect in these materials. Furthermore, the electric conductance σ and Seebeck's effect (the Seebeck's constant $\gamma = \Delta E/\Delta T$) were measured. The presence of thermic hysteresis loop was found indicating the long-lasting "dipole orientation memory" within the biomacromolecule. The data obtained are interpreted in terms of cooperative weak interactions within local domains with spontaneous polarization (ordered dipoles) dispersed in amorphous bulk material. The relevance of solid state dielectric and electric properties as well as submolecular and supramolecular structure of melanins to their physiological functions is considered.

ULTRAWEAK PHOTON EMISSION IN MODEL MELANIZATION REACTIONS D. Slawinska and J. Slawinski* Department of Physics Agricultural University 60637 Poznan, * Institute of Physics Pedagogical University, 30084 Cracow, Poland.

Oxidative polymerization reactions contributing to the biosynthesis of melanins involve free radical reactions and the ring-opening/closing steps the enthalpy of which is high enough to produce products' molecules in excited electronic states. These chemiexcitation processes manifest as spontaneous photon emission (chemiluminescence).

Using a very sensitive photoelectric device in the single photon counting mode with a high counting efficiency=number of counts/number of photons=0.1, we have investigated photon emission accompanying the following model melanization reactions: 1) the standard tyrosinase-catalyzed polymerization of i) DOPA to eumelanins, ii) 5-S-cysteinyldopa to pheomelanins, and iii) cystein + DOPA to pheomelanins (all reactions performed in 0.05M phosphate buffer at pH=6.8). 2) the autooxidation (nonenzymatic) in 0.05M phosphate buffer at pH=8.0 of: i) DOPA to eumelanin, ii) 5-S cysteinyldopa to pheomelanins, and iii) cystein + DOPA to pheomelanins. In the first step of the reaction 1) photon emission is not observed (lower than that of the buffer+substrate). In the further steps a very weak photon emission slightly exceeding that of the buffer+substrate solution, of the order of 10-100 hv $s^{-1}cm^{-3}$ is measured. In the autooxidation reaction a stronger emission in the range of 1000-3000 hv $s^{-1}cm^{-3}$ was recorded. Cysteine decreased the intensity of emission in the both reactions. Addition of chemiluminogenic probes - luminol and lucigenin as well a horseradish peroxidase enhanced photon emission. Cystein again diminished the effect of chemiluminogenic probes. The highest stimulation was exerted by the μM-mM concentrations of hydrogen peroxide.

All these data and additional tests with superoxide dismutase, catalast and spectrophotometric measurements indicate that ultraweak photon emission is associated with degradative reactions involving superoxide ion O_2 and H_2O_2 which attack the aromatic moiety of polymers (melanins).

IDENTIFICATION OF PIGMENTED SUBSTANCES IN TISSUE BY ELECTRON SPIN RESONANCE (ESR) SPECTROSCOPY

Harold M. Swartz, W. Scott Enochs, Kai Chen, and Mark J. Nilges (University of Illinois College of Medicine at Urbana-Champaign)

In a number of pathological and physiological conditions, areas containing pigment are noted in various tissues. These substances often are termed melanin, although the evidence for such a designation may be lacking entirely or limited to nonspecific histological tests such as silver staining. ESR spectroscopy provides a means to identify many of these pigments unambiguously and, in some cases, to obtain detailed information about their nature. Using a series of criteria for the intensity and other parameters of an ESR signal under various test conditions, it can be determined unambiguously whether unknown substances are melanin. Some non-melanin pigments have ESR signals, but the behavior of their signals clearly indicates they are not melanin. Examples of pigmented substances to be discussed are skin melanin, melanoma melanin, neuro-melanin, the pigment in the Dubin-Johnson syndrome, and pigments associated with the administration of minocycline.

FURTHER EVIDENCE THAT THE PIGMENT IN THE DUBIN-JOHNSON SYNDROME IS NOT MELANIN. H. M. Swartz,* K. Chen,* and J. A. Roth°. University of Illinois, College of Medicine at Urbana-Champaign, Urbana, Illinois* and Department of Pathology, Overlook Hospital, Summit, New Jersey°.

The pigment in the Dubin-Johnson Syndrome (DJS) is shown unequivocally to not be a typical melanin or closely related polymer. Using electron spin resonance studies of DJS pigment from a hepatoma, it is shown that unlike true melanins, the pigment associated with the DJS syndrome has no free radical in the absence of light. Exposure to even low levels of light induces a free radical in the DJS pigment. Previous studies did not appreciate the sensitivity to light of this pigment and therefore erroneously concluded that the DJS pigment had a permanent free radical. The light-induced ESR signal in DJS tissue has spectroscopic properties that differ significantly from typical melanins.

PHOTOOXIDATION OF INDOLIC MELANOGENIC INTERMEDIATES. [t]Ambler Thompson, *Walter H. Koch, *Miles R. Chedekel, **Edward J. Land, and [t]T.G. Truscott. *The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD. U.S.A., **Patterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, U.K. and [t]Dept. of Chemistry, Paisley College, Paisley, U.K.

The oxidation of 5,6-dihydroxyindole (DHI), 5,6-dihydroxy-2-carboxylic acid (DHI2C), and 6-hydroxy-5-methoxy-indole-2-carboxylic acid (5M6HI2C), melanogenic intermediates and metabolites, was studied by pulse radiolysis (PR), laser flash photolysis (LFP) and ESR spin trapping. Azide radical, generated pulse radiolytically, oxidized all three indoles rapidly to a mixture of two radical species: their semiquinones and their neutral radicals. LFP of these indoles indicated that while DHI photoionizes appreciably, the two carboxylic acid metabolites do not. Transient species observed during LFP of DHI2C and 5M6HI2C were identified as their semiquinones and neutral radicals, indicating photohomolysis of the -OH bond is a significant photochemical pathway. Indeed, photolysis of DHI2C and 5M6HI2C with 300 nm radiation in the presence of the nitrone spin trap DMPO confirmed the production of hydrogen atoms (H·) and provided evidence for the production of a, as yet unidentified, carbon-centered radical species. The potential photobiological significance of melanocytic indole intermediates and metabolites will be discussed.

CONTROL OF EUMELANIN-PHEOMELANIN SHIFT IN THE MOUSE MELANOCYTES

Takuji Takeuchi, Hiroaki Yamamoto and Takashi Kobunai Biological Institute and Department of Biology, Tohoku University, Sendai, Japan

Mouse melanocytes produce two kinds of melanins, eumelanin and pheomelanin. The shift in the pathways of the two melanins is controlled by the a (agouti) locus and the e (extension) locus. We have previously shown by using organ culture that eumelanin formation can be induced by α-MSH in the melanocytes of A^y/a (lethal yellow) mouse. We, however, failed to induce eumelanin formation by α-MSH in the melanocytes of e/e (recessive yellow) mouse. We then proposed that α-MSH competes with the product of the a locus at the α-MSH receptor site and that e locus controls a mechanism that determines the functionability of the α-MSH receptor.

In order to verify our hypothesis, we examined the effects of forskolin, a potent activator of adenylate cyclase in membrane, on the melanocytes of the yellow mice. Skin explants from lethal yellow and recessive yellow mice were organ-cultured for 2 days in the medium containing forskolin (10^{-6} - 10^{-4} M). Singnificant eumelanin formation was observed in the melanocytes of the lethal yellow whereas melanocytes of the recessive yellow contained exclusinvely pheomelanin. It seems likely that the e/e melanocytes possess a deficiency in α-MSH receptor-adenylate cyclase system.

Tyrosinase

DOPAMINE AGONISTS INHIBIT TYROSINASE ACTIVITY IN THE HAIR FOLLICULAR MELANOCYTES OF THE C_3H-HeA^{*vy} MOUSE **Susan A. Burchill & A.J. Thody,** Department of Dermatology, University of Newcastle upon Tyne, England.

Bromocriptine, a dopamine agonist that blocks α-MSH secretion, inhibits tyrosinase activity and eumelanin synthesis but this effect is unrelated to circulating levels of α-MSH. This therefore raises the possibility that dopaminergic mechanisms act directly to inhibit hair follicular melanocytes.

Daily administration of bromocriptine decreased tyrosinase activity and blocked the coat darkening that occurred at puberty in C_3H-HeA^{*vy} mice. It also reduced tyrosinase activity in skin explants incubated in HEPES buffered RPMI medium at 37°C. This inhibitory effect was blocked by dopamine antagonists, haloperidol and spiperone. The specific D_2 agonist LY 171555 also decreased tyrosinase activity and this was blocked by the D_2 antagonist, sulpiride. The D_1 agonist, SKF 38393, had no effect on tyrosinase activity.

These results indicate that dopamine agonists have a direct effect on hair follicular melanocytes. Dopamine agonists inhibit adenylate cyclase through D_2-receptor mechanisms and this could well explain their effects on tyrosinase activity since there is good evidence that this enzyme is regulated by the cAMP system.

MONOCLONAL ANTIBODIES TO MAMMALIAN TYROSINASE AND THEIR POTENTIAL CLINICAL APPLICATION. J.Y. Chang* and Bryan B. Fuller°, Depts. of Dermatology* and Biochemistry and Molecular Biology°, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

The characterization of tyrosinase, the rate-limiting enzyme for melanin synthesis, is of importance to the understanding of pigment biochemistry. We have produced several murine monoclonal antibodies against this enzyme. Spleen cells from Balb/c mice immunized with purified mouse tyrosinase were fused with mouse myeloma cells Ag8.563 and positive hybridomas selected by ELISA using purified tyrosinase as the coating antigen. Supernatants from E2 and B9 hybridomas immunostained the cytoplasm of both melanotic and amelanotic subclones of Cloudman S-91 melanoma cells. Immunoprecipitates of E2 or B9 antibodies and melanoma cell extracts contained active tyrosinase as shown by the production of melanin in the pellets upon addition of L-DOPA. Immunoblot staining of melanoma cell extracts by E2 antibody revealed bands at approximately 58,000, corresponding to the molecular weight of active tyrosinase. Both E2 and B9 antibodies were used to stain human tissue by the immunoperoxidase method. Strong diffuse cytoplasmic staining of human nevus and melanoma cells was observed. To assure that these monoclonals were specific for tyrosinase, sections of neurofibroma, neurilemmoma, and other non-pigment tissues were processed by similar staining methods. These monoclonal antibodies may prove useful in the diagnosis of melanoma and other pigment disorders.

TYROSINASE ACTIVITY AND ITS REGULATION DURING EUMELANOGENESIS AND PHAEOMELANOGENESIS IN THE C_3H-HeA^{*vy} MOUSE **Susan A. Burchill & A.J. Thody,** Department of Dermatology, University of Newcastle upon Tyne, England.

C_3H-HeA^{*vy} mice undergo changes in coat colour as a result of changes in the synthesis of eumelanin and phaeomelanin. In this study we have examined the regulation of tyrosinase during the synthesis of these two different melanins.

Hair growth was initiated by plucking. Hair follicular tyrosinase activity was increased at around 8-10 days after plucking but reached a higher peak level during the growth of the dark coat than the yellow coat. Moreover, during the growth of the dark coat 50% of this activity was found in the melanosomal fraction compared to only 19% during the growth of yellow hair. Administration of α-MSH, theophylline or isoprenaline during the growth of the dark hair increased tyrosinase activity and eumelanin production. Similar effects on tyrosinase activity were seen in vitro. On the other hand dopamine agonists, such as bromocriptine, inhibited tyrosinase activity both in vivo and in vitro. None of these effects were seen during the growth of a yellow coat.

These results indicate that there are differences in tyrosinase activity, its subcellular localisation and its regulation during the production of eumelanin and phaeomelanin in the hair follicular melanocytes of the C_3H-HeA^{*vy} mouse.

MAMMALIAN TYROSINASE IS AN ALLOSTERIC ENZYME AS EVIDENCED BY PH INDUCED CHANGES IN ITS PROPERTIES. Chintamaneni Chaya Devi and Abburi Ramaiah, Dept. of Biochemistry, All India Inst. of Medical Sciences, New Delhi-110029, India.

The lag in cresolase activity, its inhibition by excess tyrosine and essential requirement of dopa as co-factor were considered to be the characteristic properties of tyrosinase from many sources. These properties were absent for the partially purified tyrosinase from human skin. This led us to investigate whether the enzyme with such divergent properties are interconvertible. We demonstrate such interconversion of these two forms of enzyme for B-16 murine melanoma tyrosinase. The interconversion is brought about by decreasing pH of enzyme solution from pH6.8 to 5.0 or vice versa at 0-4°C. The enzyme at pH6.8 exists in the form with characteristics properties of tyrosinase so far known and at acidic pHs it exists in the form devoid of these properties similar to the partially purified tyrosinase from human skin. These results are interpreted in terms of an enzyme existing with allosteric site for tyrosine at pH6.8 and without it at pH5.0. According to this model binding of tyrosine at its allosteric site produces inhibition in cresolase activity which is reversed by dopa which competes effectively with tyrosine. The lag in cresolase activity was due to this effective competition by dopa that accumulates during the reaction and thus eliminating inhibition. Exposure of enzyme to pH5.0 desensitises the enzyme to allosteric inhibition and thus the lag and inhibition by excess tyrosien are lost. The physiological importance of these observations will be discussed.

REGULATIVE ASPECTS OF THE KUPFFER CELLS TYROSINASE FROM RANA ESCULENTA L.- THE EFFECTS OF MELANIN AND H_2O_2 ON THE DOPA-OXIDATION CATALYZED BY THIS ENZYME.

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In our previous studies, we showed that Kupffer Cells from Rana esculenta L. contain a tyrosinase responsible for the synthesis of their melanin content. This tyrosinase behaves as an allosteric enzyme with a negative cooperativity for its binding with the substrate. Moreover, both the degree of cooperativity and the system activity level show seasonal oscillations. We are studying the system's regulating mechanisms, and as a first step, we have considered the effects of both Dopa-melanin and of H_2O_2 on the reaction. We have observed that both substances behave as effectors by modifying the enzyme apparent affinity for the substrate. At equimolecular concentrations with the enzyme, melanin increases the enzyme affinity for the substrate, whereas at concentrations ten times higher, it decreases it remarkably. The H_2O_2 seems to always behave as negative effector for the enzyme affinity with the substrate, especially at very low concentrations. Both substances also tend to shift the negative cooperativity towards an absence of cooperativity for the substrate binding with the enzyme.

A NON-ENZYMATIC METHOD FOR ISOLATING MELANOSOMAL TYROSINASE: ORGANIC SOLVENT EXTRACTION.

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Previous techniques for isolation of melanosomal tyrosinase have usually included solubilization with trypsin and/or non-ionic detergents. There are certain draw-backs to these methods, particularly regarding the possibility of partial proteolytic digestion of tyrosinase or tyrosinase-associated proteins. We investigated the possibility of using organic solvent extraction for isolating tyrosinase from melanosomes. This technique has been used previously for the isolation of membrane-associated enzymes. Melanosomes were partially purified from tumors of the Cloudman melanoma grown in DBA/2J mice. Tumors were shear- homogenized in an aqueous buffer containing 0.25M sucrose and 1mM PMSF. Homogenates were subjected to differential centrifugations, and the resultant melanosomal fraction was applied to a discontinuous sucrose gradient. Melanosomes isolated from the gradient were pelleted by centrifugation, resuspended in a small volume of aqueous buffer, and added drop-wise to 100% acetone (-20°C). The mixture was homogenized, pelleted by centrifugation and lyopholized to dryness. The dried material was then homogenized in 100% n-butanol (4°C) centrifuged, and lyopholized. This material was resuspended in Na pyrophosphate (20mM, pH 7.4) and centrifuged at 100,000 x g for 1 hour. The supernatant containing tyrosinase activity was then further purified by DEAE, Conconavalin A, and wheat germ agglutinin chomatographic steps.

HYDROQUINONE INHIBITS TYROSINASE ACTIVITY BY ACTING AS AN ALTERNATE SUBSTRATE.

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Hydroquinone (HQ) is widely used as a skin depigmenting agent. While the exact mechanism of HQ's action is unknown, it is probably related to both the inhibition of melanin biosynthesis and the interference of the morphological development of melanocytic melanosomes. It has been shown that, under certain conditions, HQ is a substrate for tyrosinase. We now confirm, using HPLC, that HQ inhibits tyrosinase by acting as an alternate substrate. Enzymatic reactions were carried out in phosphate buffer, pH 6.8, containing 25 units of mushroom tyrosinase (EC 1.14.18.1) and 190 µM tyrosine and 3 µM DOPA. Incubations were run for varying lengths of time and stopped by direct injection onto a reverse phase HPLC column. Tyrosine and HQ concentrations in the reaction mixture were determined by HPLC. When tyrosine/DOPA was incubated with tyrosinase, tyrosine was utilized at a rate of 9.4 nmol/min. In the presence of 50 µM HQ, the rate of tyrosine utilization was slowed to 1.4 nmol/min while HQ was utilized at a rate of 2.5 nmol/min. Incubation of ^{14}C-HQ with tyrosine and tyrosinase showed the HQ was converted into a major unidentified product and to p-benzoquinone as a minor metabolite. These results support the hypothesis that HQ's mode of depigmenting action could be due to a combination of effects which include the inhibition of melanin synthesis and the tyrosinase-mediated conversion of HQ to cytotoxic products.

INTRACELLULAR PROCESSING AND CONTROL OF MAMMALIAN TYROSINASE.

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Monoclonal antibodies specifically directed against the mature form of tyrosinase (J. Invest. Derm. 85, 426) have proven to be extremely useful tools to study the rate of synthesis and degradation of this enzyme (EC 1.14.18.1). B16 F_{10} melanoma cells were grown to semiconfluence in Dulbecco's MEM with 10% fetal bovine serum, antibiotics, and non-essential amino acids. Approximately 10^7 of these cells were metabolically labeled with [^{35}S]-methionine for different pulse and chase times. The cells were harvested and solubilized with 1% NP-40 in Tris buffered saline, and the cell extracts were reacted with anti-tyrosinase monoclonal antibodies. Immunoprecipitation experiments allowed us to correlate the synthesis and expression of the nascent tyrosinase and its rate of degradation, and to determine the biological half-life of tyrosinase in melanocytes. We have also studied the responses of these cells to environmental stimulation, such as with α-MSH, using indirect immunofluorescence and immunoprecipitation techniques. The results show that tyrosinase is synthesized, and glycosylated, within melanocytes very rapidly (detectable within 30 min), but is less stable than was previously assumed (>80% of reactivity was lost within 24 hours). As for the effect of α-MSH, the stimulation of enzyme activity by α-MSH required at least 4 days to be detected.

TYROSINASE-CATALYZED BINDING OF CATECHOLS WITH PROTEINS THROUGH THE SULFHYDRYL GROUP.
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The cytotoxicity of catechols has been ascribed to covalent binding of the o-quinone oxidation products to proteins through sulfhydryl groups. The nature of the covalent binding was studied with o-quinones formed on tyrosinase oxidation of catechols. After acid hydrolysis of the reaction products, cysteinylcatechols liberated (protein-bound cysteinylcatechols) were determined by HPLC with electrochemical detection. When 0.1 mM dopa was oxidized in the presence of 0.2 mM bovine serum albumin (BSA), alcohol dehydrogenase, or isocitrate dehydrogenase, protein-bound cysteinyldopas were formed in yields of 5.4, 44, or 33%, respectively. The covalent binding was almost completely inhibited by 1 mM cysteine or 1 mM ascorbic acid, but 10 mM lysine had no effect. These results unambiguously demonstrate that dopaquinone can bind with proteins mostly through sulfhydryl groups.

The reactivities of the oxidation products of other catechols are being compared with that of dopaquinone. Preliminary results indicate that o-quinone forms of dopamine, N-acetyldopamine, pyrocatechol, and 4-methylcatechol are much more reactive with BSA than dopaquinone while 5-S-cysteinyldopaquinone is least reactive.

SITE-SPECIFIC MUTAGENESIS OF THE STREPTOMYCES TYROSINASE GENE: IDENTIFICATION OF HISTIDINE 62 AS A COPPER LIGAND
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Tyrosinase is a copper-containing monooxygenase catalyzing the formation of melanin pigments. The active site of the enzyme consists of a coupled copper pair which interacts with O_2 and organic substrates. Chemical and spectroscopic studies have shown, that the tyrosinase copper site is very similar to that of the O_2-binding hemocyanins. Moreover, amino acid sequence comparison showed the two proteins to share a highly conserved region with three invariant histidine residues known to be ligands to Cu(B) in Panulirus interruptus hemocyanin by X-ray cristallography. In contrast, however, the sequence homology for the region of Cu(A) is rather small. To obtain further insight into the ligand environment of Cu(A), the structural gene of Streptomyces glaucescens tyrosinase was modified by site-specific mutagenesis replacing histidine 62 by asparagine. The mutant gene was expressed in a tyrosinase deficient strain of S. glaucescens. Only white colonies were found on indicator plates for melanin production while the wild-type gene typically yields black colonies. The enzyme was purified to homogeneity and found to be totally inactive. A comparison of the chemical and spectroscopic properties of the mutant and the wild-type enzyme lend strong support for histidine 62 as a ligand to Cu(A) in S. glaucescens tyrosinase.

MOLECULAR CLONING OF cDNA FOR HUMAN TYROSINASE.
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A λgt11 cDNA library of normal human melanocytes was screened with antibodies directed against purified hamster tyrosinase. Three clones which gave a strong positive signal were isolated from 500,000 independent plaques. cDNA inserts of the clones were cross-hybridized with each other, indicating that they were from the same mRNA species. mRNA corresponding to the putative tyrosinase cDNA was expressed specifically in melanocytes. The cDNA was hybridized to approximately 2.4 kb mRNA species of both human and mouse melanocytes. The abundance of mRNA corresponding to the cDNA intimately reflected the tyrosinase activity and melanin content in various human and mouse melanocytes. Data will be presented to establish that the cDNA clones contained coding sequence of human tyrosinase.

TYROSINASE ACTIVITY IN THE FIRST COAT OF AGOUTI AND BLACK MICE.
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Tyrosinase activity was compared in black and agouti mice of varying ages (4-12 day-old). Differences in activity were found to be maximal at the time of yellow pigment synthesis in agouti mice in both the hair and skin. Although histological examination showed that the number of dopa-positive melanocytes is lower in the agouti hair bulb than in black mice, this did not account for the differences in enzyme activity observed. The level of SH-compounds in the hair bulb was examined and found to be elevated in agouti pigment cells at the time of phaeomelanin formation. It was shown that sulphydryl compounds such as cystein and glutathione have an inhibitory effect on the enzyme and it is possible that the elevated levels of SH-compounds are responsible for a reduction in tyrosinase activity in agouti mice. In agouti hair bulb this can be reversed, in vitro by treatment with copper. These observations suggest that SH-compounds as cysteine seems to play an inportant role in phaeomelanin formation.

TYROSINASE CATALYSED OXIDATION OF MONOPHENOLS
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The reaction mechanism for tyrosinase-catalysed oxidation of monophenols requires the presence of diphenolic species to recruit the met-form of the enzyme to the active monophenolase by reduction of the cupric ions at the active site of the enzyme. The met-form of the enzyme is generated in the oxidation of diphenols to the corresponding quinone. The lag phase of monophenol oxidation can be explained because a large amount of native enzyme is in the met form, which requires reduction by diphenolic products to form the deoxy-form in which it binds oxygen to form the active monophenolase. The monophenol competes for met-tyrosinase, hence reducing the rate of met-form recruitment at high substrate concentration. The lag phase can be shortened by exogenous reductants (eg dopa) due to increased recruitment of met-tyrosinase to the active monophenolase. It is unlikely that the endogenously generated diphenol will leave the active site at which it is generated and diffuse away to recruit met-enzyme; a diphenolic species generated subsequently is more likely to perform the reduction. Cyclodopa or 5,6-dihydroxyindole are candidates in tyrosine oxidation, being oxidised to dopachrome and indole 5,6-quinone respectively. Autoreduction of the quinone by side chain cyclisation is not possible in the case of the oxidation of 4-hydroxyanisole. Dimers and oligomers of the primary oxidation product, 4-methoxy ortho benzoquinone are suggested as possible met-tyrosinase recruiting species.

THE RELATION OF COAT COLOR IN TYROSINE HYDROXYLASE AND DOPA OXIDASE ACTIVITY IN C57BL/6J MICE. D Townsend, D Olds, CJ Witkop, RA King. U of MN, Mpls, MN.

The tyrosine hydroxylase (TH) and dopa oxidase (DO) functions of tyrosinase were determined in mice with different coat colors to investigate the role of these enzymatic functions on coat color production. The mouse mutants were the brown, recessive yellow, viable yellow, pink-eye, and standard nonagouti, all on the C57BL/6J background. TH activity was determined with a tritiated tyrosine assay. DO activity was determined by the HPLC measurement of 5-S-cysteinyldopa formation in a reaction mixture containing dopa and cysteine. The results are:

Mice	TH nmol/hr/25ul	DO ng/20ul	TH/DO Ratio
Standard	6.81	2.67	2.55
Brown	16.86	16.50	1.02
Recessive yellow	0.88	0.90	0.98
Viable yellow	0.11	0.50	0.21
Pink-eye	5.30	1.85	2.86

Compared to the standard, both activities of tyrosinase were high in the brown and low in the yellow mice, but the relative amounts were different. In brown, the DO increased 6.2X while the TH increased 2.5X over the standard. In recessive and viable yellow, the reduction was greater in TH than in DO when compared to the standard. There appears to be a correlation between coat color and the different functions of tyrosianse.

GENERATION OF AMELANOTIC CELLS FROM A HUMAN MELANOTIC CELL LINE: ABSENCE OF TYROSINASE ACTIVITY IS CORRELATED WITH DEPRESSED CYCLIC AMP BINDING PROTEINS.
R.M. Niles, B.P. Loewy, and D.DiSorbo, Boston Univ. School of Medicine, Boston, MA. 02118

Nel-Ml cells were originally isolated from a human melanotic tumor. This cell line has been adapt- to grow in serum-free medium and produces endogenous growth factors. During the course of in vitro culture the cells became amelanotic. At this stage MSH does not stimulate melanogenesis nor does it inhibit growth, although it markedly increases cAMP levels. Since we have found that an MSH-resistant mutant of B16 mouse melanoma had depressed levels of tyrosinase and cAMP-dependent protein kinase (cAPK), we investigated these parameters in the human cells. At high densities, the NEL-Ml cells were amelanotic as evidenced by a completely white cell pellet. Tyrosinase activity in extracts from these cells was below the detectable level of our assay. Using the photoreactive analog 8-azido-32P-cAMP, two proteins at Mr 49,000 and 54,000 corresponding to the regulatory sub units of type I and II cAPK respectively were found to specifically bind the ligand. The amount or binding of the 49,000 MW protein was markedly reduced in the amelanotic cells. These results suggest that cAPK may regulate basal and MSH stimulated tyrosinase activity. It is interesting to note that this suppression of melanogenesis is not permanent since these amelanotic cells form pigmented tumors when injected into nude mice. Supported in part by GRS NIH RR05487 -23 (D.D.) and NCI CA32543 (R.N.)

pH DEPENDENT INTERCONVERTIBLE FORMS OF TYROSINASE IN INTACT HUMAN MELANOSOMES.
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Tyrosinase catalyses the hydroxylation of tyrosine to 3,4-dihydroxy phenyl alanine (dopa) and oxidation of dopa to dopaquinone. B-16 murine melanoma tyrosinase with characteristic lag, requirement of dopa as essential cofactor could be converted to a form devoid of these properties by lowering the pH of the enzyme solution to pH5.0. But so far this form of enzyme was never observed when the tyrosinase was solubilized at pH6.8. But actually this form of enzyme may exist in the intact melanosomes since melanosomal pH may be acidic and during extraction of the enzyme in buffer at pH6.8, this form of enzyme might have been converted to the other form. Therefore, in the present study the enzyme from the human melanosomes was solubilized by sonication of melanosomal fraction at various pHs and time course of its cresolase activity was tested both at pH5.2 and 6.8 at inhibitory concentration of tyrosine in order to see whether tyrosine uninhibitable form could be detected under any of these conditions. The results show that the water detergent extracted enzyme exhibits tyrosine uninhibitable form of tyrosinase when its activity was estimated at pH5.2 while it shows estimated at pH6.8. The pH5.2 extracted enzyme exists again in both the forms, while the pH6.8 extracted enzyme exists in only tyrosine inhibitable form. These two forms of the enzyme could be interconverted by appropriate change in pH of enzyme solution. It is speculated that the enzyme in the intact melanosomes exist in at least two interconvertible forms depending on the pH of the melanosomes.

QUANTITATION OF HAMSTER SERUM TYROSINASE BY RADIOIMMUNOASSAY

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Tyrosinase /EC 1.14.18.1/ is responsible for the melanin synthesis in pigment cells. Melanosomal tyrosinase was purified from hamster melanoma to homogeneity and radioiodinated with ^{125}I. Using the labeled enzyme and polyclonal rabbit antibodies to hamster tyrosinase, we quantitated the enzyme by radioimmunoassay. The serum tyrosinase levels were found to be about 0.24 ug and 1.14 - 4.48 ug per ml of serum in normal hamsters and melanoma-bearing hamsters, respectively. Very low levels of tyrosinase were detected in the sera from melanoma-bearing mice in which high enzyme activity were found, indicating thus a high specificity of the assay. The direct quantitation of tyrosinase protein by radioimmunoanalysis seems to be specific and sensitive, and, therefore, of importance since the tyrosinase inhibitor/s/ abundantly present in pigmented tissues may hamper the estimation of the enzymatic activity, particularly in the crude samples.

Color Change-Hormones

CHARACTERISATION OF HISTAMINERGIC RECEPTORS ON ISOLATED FISH MELANOPHORES

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Fish melanophores have rarely been studied for the effects of histamine and as such its role in chromatic science is still sceptical. In order to investigate the role of autacoids and their receptors, the isolated scale melanophores of an Indian fish Ophiocephalus punctatus were selected. The responses of the melanophores in control saline and in test substances were recorded as mean melanophore size index. Histamine per se (10^{-6} to 6.4×10^{-5} g/ml) displayed a poweful dose dependent melanophore dispersal effect which was completely blocked by both mepyramine and metiamide, the antagonism of the former was more effective. This suggestive of the fact that both histaminergic receptors of H_1 and H_2 type are present on the melanophores of this species, however H_1 seem to be dominent and more evolved. 2-Methyl histamine, (2-MH, 10^{-6} - 6.4×10^{-5} g/ml) caused an unexpected melanophore aggregation which could not be blocked by mepyramine or metiamide. This responses probably due to indirect release of catecholamines. 4-methyl histamine (4-MH, 10^{-6} - 6.4×10^{-6} g/ml) produced a powerful melanophore dispersal effect, which was rnot blocked by metiamide, on the other hand it was greatly potentiated. It is proposed that 4-MH unorthodoxly stimulates the 'spare' H_1 receptors, and causes the strange effect. It is concluded that both H_1 and H_2 receptors are present however, they are not clearly differentiated and do not show advanced specificity as observed in mammalian tissues.

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EFFECT OF DICHLOROVOS (DDVP) ON THE ISOLATED MELANOPHORES OF OPHIOCEPHALUS STRIATUS

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Organophosphorous compounds are well known anticholinesterase agents in various mammalian tissues, however little is known about their effects on fish melanophores. In the present paper we have studied the effects of dichlorovos, 0-0 dimethyl 2-2 dichlorovinyl phosphate (DDVP) on the isolated scale melanophores of a fresh water teleost fish Ophiocephalus striatus. Mean melanophore size index (MMSI) was recorded of both, control as well as drug treated melanophores. It was found that the MMSI increased instantly from a control value of 3.5 ± 0.5 to 6.0 ± 0.5 upon incubation of the cells in DDVP concentration of 5×10^{-9} g/ml. A dose dependant melanophore dispersal response was obtained and the maximum concentration of 6.4×10^{-7} g/ml induced a powerful dispersal effect (MMSI = 11.5 ± 0.5). This effect could be completely blocked by both scopolamine and atropine, in low concentrations. The effect of DDVP on the responses of melanophores to acetylcholine was also studied, it was found that the dispersal response of acetylcholine was greatly potentiated. It is concluded that DDVP acts as strong anticholinesterase agent, and the melanophore dispersal response in this species is mediated by cholinergic receptors of muscearinic type.

(Research Grant from Department of Atomic Energy BARC, Government of India to first author is acknowledged).

THE INVOLVEMENT OF HISTAMINE RECEPTORS IN SKIN DEPIGMENTATION OF INDIAN FROG, RANA TIGRINA

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The isolated dorsal region skin of Indian frog, **RANA TIGRINIA** has been used in the present study, inorder to investigate the involvement of histamine receptors in epidermal melanophore responses. Histamine per se in a dose range of 10^{-5} to 10^{-8} g/ml caused a strong dose-dependent melanophore aggregating effect. This response was blocked by mepyramine as well as by cimetidine. The synergistic effect of both the receptor antagonists was more pronounced and long lasting. It is proposed that both type of histaminergic receptors - H_1 and H_2 are present on the skin melanophores of **Rana tigrina**, and they mediate melanophore aggregation causing skin depigmentation.

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A TELEOST SKIN BIOASSAY FOR MELANOTROPIC PEPTIDES*.

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A teleost (the eel,Synbranchus marmoratus) skin bioassay for melanotropic peptides and other agonists is described. Unlike previous teleost assays that generally monitor or observe individual melanophores, this objective assay monitors the reflectance changes of large intact pieces of skin. Since melanosomes within most teleost melanophores are dispersed, the present assay provides a method for measuring the response of integumental melanophores to melanosome aggregating agents such as MCH, a putative melanin concentrating hormone. This bioassay is sensitive to MCH at a concentration as low as 10^{-12}M. Because of the magnitude of this lightening response, four-point dose-response curves can be obtained. Skins lightened by MCH can then be used for bioassay α-melanotropin (α-MSH) and related analogs. This bioassay is sensitive to α-MSH at a concentration of 10^{-10}M. This bioassay is unique in providing a method for determining the biological activities of melanotropic peptides with opposing actions.

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IN VITRO ANIMAL AND HUMAN CADAVER SKIN MODELS FOR PERCUTANEOUS DELIVERY OF MELANOTROPIN ANALOGS

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Transdermal (percutaneous) delivery of superpotent analogs of melanocyte stimulating hormone (α-MSH) has been achieved in vitro in a rodent and a human cadaver skin model. These studies are precursors to clinical trials using these melanotropins in the treatment of certain hypopigmentary diseases and in stimulating melanogenesis in normal skin.

The passage of [Nle^4, D-Phe^7]-α-MSH analogs through mouse skin in vivo causing a shift from pheomelanogenesis to eumelanogenesis has been demonstrated by other investigators and has now been confirmed by in vitro studies. Rat skin did not demonstrate similar permeability. Since human skin is undoubtedly the best model for testing transdermal delivery, and since the stratum corneum has been shown to be the greatest barrier to most drugs, studies have been designed using human surgically excised and cadaver skin with intact stratum corneum to test penetration of melanotropic peptides. The α-MSH analogs in various vehicles (e.g. polyethylene glycol, propylene glycol) have been applied to skin obtained from a variety of anatomical sites and mounted on specially designed penetration cells. The transdermal passage of the peptides has been measured by frog skin bioassay of the subdermal collection fluid. [Nle^4, D-Phe^7]-α-MSH analogs have been demonstrated to traverse intact human stratum corneum and dermis in measurable quantities.

IN VIVO BLOOD KINETICS OF α-MELANOCYTE STIMULATING HORMONE (α-MSH) AND A SUPERPOTENT ANALOG

Don, S., Hadley, M.E., Dawson, B.V. and *Hruby, V.J., Depts. of Anatomy and *Chemistry, University of Arizona, Tucson, AZ USA

Both α-MSH and certain potent analogs are capable of inducing eumelanogenesis in yellow (C57BL/6JA^Y) mice when injected or delivered transdermally. One analog, [Nle^4, D-Phe^7]-α-MSH, is highly resistant to degradation by serum enzymes. The analog may remain intact longer in the circulation. This has been confirmed experimentally using a Sprague-Dawley rat model. α-MSH or [Nle^4, D-Phe^7]-α-MSH was injected intraperitoneally (10^{-4} molar; 0.1 ml/100 g body weight), and the rats were bled at various times thereafter with serum collected and frozen for subsequent bioassay to measure serum melanotropin levels.

[Nle^4, D-Phe^7]-α-MSH could be detected in biologically active form in the systemic circulation of the rat four times longer than the natural hormone. These results confirm the validity of other in vitro studies which have shown MSH to be rapidly degraded by serum enzymes. The analog could not be detected after four hours post intraperitoneal injection (possibly as a result of sequestration in tissues, excretion in urine, or protein binding as opposed to enzyme degradation).

The extended half-life of this analog compared with the native hormone is of considerable clinical relevance in the anticipated use of these peptides in stimulation of melanogenesis in human skin and in the treatment of some hypopigmentary disorders.

OCCURRENCE OF CHOLINOCEPTORS IN THE MELANOPHORE OF SOME CATFISHES AND THE PHYLOGENY OF SILURIFORMES. R. Fujii, H. Kasukawa and N. Oshima. Department of Biology, Faculty of Science, Toho University, Funabashi, Chiba 274, Japan.

We have found two teleost species in the family Siluridae (order: Siluriformes), in which the neurotransmission to melanophores is strangely cholinergic, being mediated by muscarinic cholinoceptors. Quite lately, furthermore, we discovered cases among mailed catfishes (Corydoras spp., Callichthyidae), in which, although the neuro-transmission is orthodoxly adrenergic, the cells possess extra muscarinic cholinoceptors of unknown physiological significance, which also mediate melanosome aggregation. A further survey broadly into many catfish families has diclosed that at least some species belonging to Bagridae and Pimelodidae also possess melanophores endowed with the cholinoceptors. Thus, in three out of 13 families examined to date, there exist melanophores of "Corydoras" type. Although the cells possess distinct cholinoceptors, the neuro-melanophore transmission was by no means cholinergic. We presume that the fishes having those melanophores are of the transitional type from common and orthodox fishes to those of Siluridae, when we consider the latters are of more evolved group. Evolution of the reverse direction might also be speculated, if silurids were the prototype of Siluriformes. Phylogeny of the fishes within the order Siluriformes is discussed on the basis of the present data and the past knowledges about the pigment cell receptors.

ALPHA-MELANOCYTE STIMULATING HORMONE RECEPTORS ON HUMAN MALIGNANT MELANOMA CELLS IN CULTURE. Ghanem,G. Libert,A. et al. Jules-Bordet Inst. Brussels, Belgium

Alpha-Melanocyte Stimulating Hormone (α-MSH) is known to be the main pigmentation hormone. Nevertheless, its mechanism of action at the human cell receptor level has not been clearly demonstrated yet. MSH binding assays appeared to be difficult to achieve and showed poor reproducibility. Such studies were mainly reported by three groups : 1) Varga J.M. (1974) and Fuller B. (1980) with β-MSH on Cloudman mouse melanoma cells; 2) Halaban R. (1983) with β-MSH on normal and malignant melanocytes; 3) and finally Eberle A.N. (1979) with α-MSH on Cloudman mouse melanoma cells while other researchers failed to reproduce the same studies on human melanoma cells (Wallevik K., 1983).

The aim of our study was to design a reproducible α-MSH receptor binding assay on cultured human malignant melanoma cells and to assess the receptor(s) specificity and affinity. We used high specific activity 125 iodine α-MSH (+2Ci/μg) on two non-synchronized human melanoma cell lines (HBL,SCL) established in our laboratory. The tracer specifically bound to melanoma cells was determined using the isotopic dilution technique with unlabelled α-MSH, β-MSH, β-LPH, γ-MSH, $ACTH^{(1-39)}$, $ACTH^{(1-24)}$, $ACTH^{(4-10)}$, and other α-MSH non-related peptides (concentrations ranging from 10^{-11} to 10^{-6}M). Affinity constants calculated from Scatchard plots averaged 10^{7}l/mole for both cell lines.

MSH EFFECTS ON THE SYNTHESIS, ACTIVATION AND DEGRADATION OF TYROSINASE IN MELANOMA CELL CULTURES. Bryan B. Fuller, Dept. of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Cloudman S-91 mouse melanoma cells respond to MSH by demonstrating an increase in tyrosinase activity. We have previously shown that this response is dependent upon transcription and translation. Immunoprecipitation analysis of ^{3}H-leucine pulse-labeled tyrosinase has shown that although enzyme activity may increase more than 90 fold in cells treated with MSH for 6 days, tyrosinase synthesis rates increase by, at most, 3 fold. Evidence for the presence of inactive (or less active) enzyme molecules has been obtained by competitive ELISA analysis, immunoblots of cell extracts from control and MSH-treated cells and from experiments showing that unstimulated cell cultures can undergo a "self-activation" process resulting in large increases in tyrosinase activity. To determine if tyrosinase degradation rates may be altered in MSH-treated cells, melanoma cultures were incubated in medium containing ^{35}S-methionine for 48 hrs., the label removed and cells incubated in unlabeled medium in the presence or absence of MSH for 72 hrs. Duplicate flasks were removed at 24 hr. intervals and tyrosinase immunoprecipitated. Results from these studies indicate that the large increase in tyrosinase activity in cells treated with MSH cannot be explained by changes in the degradation rate of the enzyme but rather by an MSH effect on both the synthesis and activation of tyrosinase.

TARGETING OF HUMAN MALIGNANT MELANOMA WITH ALPHA-MELANOCYTE STIMULATING HORMONE (α-MSH) COUPLED TO MELPHALAN. Ghanem,G., Scarso, A. et al. Jules-Bordet Inst.,Brussels, Belgium

Previous works from our laboratories have shown that synthetic iodinated α-MSH binds to human malignant melanoma cell lines through a specific receptor. In addition, significant binding of the labelled hormone to melanoma metastases in patients was demonstrated.

In order to target malignant melanoma, we designed a drug coupled hormone. The alkylating agent L-Phenyl Alanine Mustard (Melphalan) was coupled by covalent bond on the Lysine at the 11 position of the amino-acid sequence of synthetic α-MSH. The product was purified to 95% and found to be stable at 4°C in a dried powder form. When assayed in an optimalized radio receptor assay on cell lines, it was found to have higher affinity for the receptor that the native hormone. No binding was found to control fibroblasts nor to carcinoma cells.

Cytotoxic effect was studied in vitro using ^{3}HTdR uptake and was six times higher with drug-coupled hormone than free Melphalan. No cytotoxicity was noticed on P388D1 cells using the synthetized complex while the free drug was still very active. Biodistribution and kinetic studies are underway.

We can conclude that α-MSH-Melphalan conjugate shows enough interesting properties to be considered for future drug targeting studies in human melanoma.

HORMONAL REGULATION OF PHENOLOXIDASE AND DOPA DECARBOXYLASE IN MELANIZATION OF MANDUCA SEXTA.
Kiyoshi Hiruma and Lynn M. Riddiford, University of Washington, Seattle, WA 98195, USA.

During a larval molt the absence of juvenile hormone (JH) around the time of head capsule slippage (HCS) causes the deposition of premelanin granules containing prophenoloxidase (pro-PO) into the newly forming cuticle 13 hr later. When the ecdysteroid titer declines, the enzyme is activated and melanization occurs 3 hr before ecdysis. Treatment of the cuticle with 0.1% SDS followed by electrophoresis showed 4 granular PO (110, 105, 100 and 94 kD) unique to cuticle containing premelanin granules and one (68 kD) common with cuticle lacking the granules. The pro-PO appeared in the epidermis at 10 hr after HCS, then disappeared from this tissue when it first appeared in the cuticle at 13 hr after HCS. Incorporation of ^{3}H-leucine into the pro-PO stopped shortly before its activation. A second enzyme, dopa decarboxylase (DDC), is required for melanization since in this insect dopamine is the primary precursor for melanin. The level of DDC activity is two-fold higher in melanizing larvae due to the absence of JH just after HCS. Moreover, the increase in DDC synthesis at the end of the molt can be inhibited by 20-hydroxyecdysone (20-HE) both in vivo and in vitro. Two putative genomic clones for the Manduca DDC gene have been isolated and are being mapped in order to study its regulation by 20-HE and JH. Supported by NSF DCB 80-11152, DCB 85-10875 and NIH AI 12459.

INDUCTION OF MELANIZATION WITHIN HAIR BULB MELANOCYTE IN CHINCHILLA MUTANT BY MELANOGENIC STIMULANTS
Genji Imokawa, Yukihiro Yada, Masako Mizoguchi and Yoshiaki Hori, Tochigi Res. Lab., Kao Corporation, Tochigi, Department of Dermatology, Teikyo Univ., Tokyo and Yamanashi Medical College, Yamanashi, Japan

Hair bulb melanocytes in chinchilla mice(genotype a/a, c^{ch}/c^{ch}, strain PW) which has white hair and pink eyes, express melanosome production and tyrosinase activity, but possess no substantial melanin formation in the melanosomes. We therefore investigated the mechanisms underlying the lack of melanin formation by analyzing the effect of exogenous melanogenic stimulants such as theophylline(Tp), dibutyryl cyclic AMP (db-cAMP) and α-MSH on the melanogenesis in hair bulb melanocytes. Skin explants excised from the dorsa of chinchilla or lethal yellow mice(C57BL/6J, A^{y}/a) at 7-9 days of age were cultured in the presence of Tp(2mM), db-cAMP(2mM) or α-MSH(1ug/ml). After 2-5 days, the melanin formation was induced in hair bulb melanocytes of chinchilla mutant in response to both Tp and db-cAMP whereas α-MSH did not cause the new melanin formation. In contrast, yellow mutant increased the melanin formation in response to all stimulants with an order of db-cAMP > α-MSH > Tp. Quantitative analysis of the induced melanin has revealed that in chinchilla mutant Tp caused the 12 fold increase in the formation of eumelanin on day 5, while pheomelanin also increased up to 6 fold. These results suggest the defect of tyrosinase activation system within hair bulb melanocytes in chinchilla mutant.

NERVOUS CONTROL OF MOTILE IRIDOPHORES OF ODONTOBUTIS OBSCURA (TELEOSTEI)
Tetsuro Iga, Ikuo Takabatake and Akira Matsuno, Department of Biology, Faculty of Science, Shimane University, Matsue, Japan.

Iridophores in the dermis of the skin of a freshwater goby, Odontobutis obscura are motile. They respond to melatonin and norepinephrine with dispersion of reflecting platelets within the cell and to MSH with concentration of them in the centrosphere. The movements, as compared with those of melanophores, are remarkably slow. Electrical stimulation caused platelet dispersion within the cell. 10^{-6}M TTX blocked its effect without inhibiting responsiveness of the cells. The electrical stimulation, however, failed to induce platelet dispersion of iridophores in scales excised from chemically denervated fishes. Experiments on uptake of ^{3}H-norepinephrine (^{3}H-NE) showed nerve fibers labeled with ^{3}H-NE passing over the iridophores. The effect of electrical stimulation was inhibited with alpha adrenergic antagonists, but not with beta ones. The conclusions are reached: (1) The motile iridophores are under the nervous control in addition to the hormonal control. (2) The nerves are of the sympathetic adrenergic system. (3) Adrenergic receptors on the iridophore membrane mediating the platelet dispersion caused by the transmitter are of the alpha type.

EFFECT OF HYPOTHALECTOMY ON THE DIFFERENTIATION OF MSH CELLS IN THE PITUITARY GLAND OF TOAD TADPOLES.
S. Kikuyama, K. Kawamura, H. Inaco and B. Jenks, Dept. Biol., Sch. Educ., Waseda Univ., Japan and Dept. Zool., Catholic Univ. Nijmegen, The Netherlands.

Experiments were conducted to see whether differentiation of MSH cells in Bufo japonicus formosus larvae is dependent on hypothalamus. Primordium of posterior hypothalamus was surgically removed at open neurula stage. After 3 weeks, development of MSH cells was studied immunohistochemically using anti α-MSH serum.

In the specimens lacking posterior hypothalamus, epithelial hypophysis was located away from the normal site and no immunoreactive MSH cells were present (n=14). In the specimens of which posterior hypothalamus developed and epithelial hypophysis was in contact with infundibular stalk, MSH cells were invariably present (n=14). Whereas, immunoreactive prolactin cells were present in all cases.

It is concluded that posterior hypothalamus plays an important role for the migration of pituitary primordium to the ordinary position and for the differentiation of MSH cells.

SPECIFICITY OF AN α-MSH RADIOIMMUNOASSAY FOR [NLE^4, D-PHE^7]-α-MSH, A SUPERPOTENT α-MELANOTROPIN
K.L. Kreutzfeld, S.T. Wilson and J.T. Bagnara, Dept. Anatomy, Univ. of Arizona, Tucson, AZ, USA

A commercial radioimmunoassay for α-MSH (α-MSH-RIA) marketed by ImmunoNuclear, Inc. (Stillwater, MN) contains polyclonal antibodies specific for -MSH and exhibits low cross-reactivity with other related molecules of the POMC family. In addition to α-MSH, the native melanotropin, superpotent [Nle^4, D-Phe^7]-substituted analogs are being used and quantitation of these analogs has become important. In the absence of antisera to these analogs, the capacity of the α-MSH-RIA kit to detect [Nle^4, D-Phe^7]-α-MSH and Ac-[Nle^4, D-Phe^7]-α-MSH$_{4-10}$-NH$_2$ (courtesy V.J. Hruby) was analyzed by constructing standard curves for the two analogs in a heterologous (analog vs. labeled α-MSH) system. The -MSH-RIA kit exhibits a sensitivity for [Nle^4, D-Phe^7]-α-MSH that is linear (logit/ln plot). The detection limit of the kit for α-MSH is 2-18 pg/tube in our hands, whereas that for [Nle^4, D-Phe^7]-α-MSH was 0.5-2.0 pg/tube. Sensitivity of the kit for [Nle^4, D-Phe^7]-α-MSH seemingly correlates with its potent (10-10,000-fold more potent than α-MSH) pigmentary activities but cannot be related to it because Ac-[Nle^4, D-Phe^7]-α-MSH$_{4-10}$-NH$_2$ is also superotent yet has low cross-reactivity with these polyclonal antibodies. Even though the assay system is heterologous, the standard curves are repeatable and linear, making the ImmunoNuclear α-MSH-RIA system useful for the detection of [Nle^4, D-Phe^7]-α-MSH, as well as α-MSH.

MODULATION OF MELANOSOME DISPERSION BY PROTEIN KINASE C **Angela M. Lucas, Sam Shuster & A.J. Thody**, Department of Dermatology, University of Newcastle upon Tyne, England.

Melanosome dispersion is mediated by the enzyme adenylate cyclase (AC) through increases in cAMP and protein kinase A. We have suggested that protein kinase C (PK-C) interacts with PK-A, to modulate the melanophore response. We now examine at which level this interaction occurs in melanophores of the lizard Anolis carolinensis. The phorbol ester TPA (3-30nM), which stimulates PK-C, potentiated the response of α-MSH, dose- dependently. TPA (16nM) also potentiated the response of forskolin (<0.3μM), which activates the catalytic moiety (C) of AC, the subunit linked to MSH receptors via a GTP-binding protein, N_s. However, TPA (<30nM) had no effect on the response to 8-bromo cAMP (<2.5mM) indicating that its site of modulation precedes cAMP synthesis. Clonidine (5μM) an α_2-receptor agonist which inhibits AC by the uncoupling of N_s and C, decreased α-MSH potency and inhibited the TPA-potentiated α-MSH response even further. It also inhibited forskolin, and the TPA- potentiated forskolin response but had no effect on the cAMP response. These results suggest that PK-C and AC interact at a point preceding cAMP synthesis, and that the subsequent signal can be inhibited via the α_2-mediated uncoupling of AC. We propose that the interaction of PK-C and PK-A is at the level of N_s linkage with C of AC, and that PK-C-mediated phosphorylation of one or both of these subunits may activate AC and thus potentiate the response to melanophore agonists.

STIMULATION OF MURINE FOLLICULAR MELANOGENESIS BY SYSTEMIC AND TOPICAL MELANOTROPINS. Norman Levine, Mac E. Hadley, Athena Lemus and Victor J. Hruby, Departments of Medicine (Dermatology), Anatomy, and Chemistry, Univeristy of Arizona, Tucson, Arizona, U.S.A.

The relative effects of α-MSH on follicular melanogenesis were studied in the mouse. The native hormone as well as [Nle^4, D-Phe^7]-α-MSH and related fragment analogues Ac-[Nle^4, D-Phe^7]-α-MSH$_{4-11}$ -NH$_2$ and Ac-[Nle^4, D-Phe^7]-α-MSH$_{4-10}$ -NH$_2$ were serially injected subcutaneously or applied topically to the dorsal skin of C57BL/6JA^y yellow mice whose hair follicles normally produce pheomelanin but which are capable of switching to eumelanin synthesis after appropriate stimulation.

After subcutaneous injection, α-MSH stimulated follicular eumelanin synthesis, manifested by dark brown pigment in the hair bulbs, electron microscopic evidence of eumelanosomes and clinical darkening of hairs which were allowed to grow out after treatment. A 100-fold lower concentration of the synthetic hormones produced these same effects. Topical application of the melanotropins also produced follicular eumelanogenesis both locally and at distant sites suggesting systemic absorption of the peptides. The synthetic derivates produced eumelanin at a concentration 100,000 times lower than α-MSH.

These results demonstrate that peptide hormones can be delivered percutaneously and that potent synthetic melanotropins are capable of stimulating follicular melanogenesis.

OCCURRENCE OF FINE FILAMENTS BETWEEN ISOLATED PIGMENT GRANULES FROM MIDGUT ERYTHROPHORES OF CARIDINA DENTACULATA. M. Miyawaki, T. Tsuruda, I. Yoshioka, Department of Biology, Faculty of Science, Kumamoto University, Kumamoto, Japan.

When the erythrophores was ruptured, cytoplasmic matrix and pigment granules, which are linearly arranged, were flown out. By an electron microscopic observation, each granule is connected with adjacent one with fine filament. All of these observations were made in Locke's solution. But, when the procedure was performed initially in distilled water, fine pigment grain in the pigment granules was dissolved. And it is clear that the filaments penetrate through the pigment granules themselves.

PROFILES OF CLOUDMAN S91 TYROSINASE ACTIVITY INDUCED BY α-MELANOTROPINS IN MICROTITER-WELL ASSAY SYSTEMS
Marybeth Mulcahy, Kristie L. Kreutzfeld and Mac E. Hadley, Dept. of Anatomy, Univ. of Arizona, Tucson, AZ, USA

A modification of the Pomerantz tyrosinase assay has been utilized by many investigators to quantitate melanogenesis in vitro by determining the amount of ^3H_2O released into incubation media during the conversion of ^{3}H-tyrosine to L-DOPA. The enzyme activity, tyrosinase, is then expressed per number of cells, ug protein, or DNA. We have attempted to utilize a scaled-down version (1/10th to 1/5th) relative to the previously published tyrosinase assay system frequently performed in 25 cm^2 flasks. We present the 24, 48 and 72 hour profiles of α-melanotropin induced tyrosinase activity as determined in 2.5 and 5.0 ml microtiter wells. As described earlier, the potent melanotropin analog, [Nle^4, D-Phe^7]-α-MSH, is 100-fold more active in terms of minimal effective concentration than is the native hormone, α-MSH (10^{-11}M vs 10^{-9}M, respectively) after 24, 48 and 72 hours in the presence of the hormones, using 25 cm^2 flasks. In our initial experiments, these potency relationships are maintained in the proportionately smaller microtiter well assay systems in which data are expressed as units of tyrosinase activity per ug protein. The microtiter well variations of the modified Pomerantz assay yield consistent relative potency results as long as the Cloudman S91 melanoma cells are at an optimum density (5 X 10^4 cells/cm^2) at the time of treatment.

THE ROLE OF CALCIUM IN LIGHT RESPONSE OF ORYZIAS MELANOPHORES
S.Negishi, Dept. Biol., Keio Univ., Yokohama, Japan

Light-induced melanosome dispersion of Oryzias cultured melanophores is completely inhibited by Ca^{2+} antagonists, La^{3+} (0.2 mM), verapamil (0.3 mM) and papaverine (0.3 mM), but slightly by ruthenium red (4 mM). As the former drugs are thought to block Ca^{2+} transport in plasma membrane, it seems that light-response of melanophores depends on Ca^{2+} flux in plasma membrane. Theophylline-induced melanosome dispersion is not prevented by Ca^{2+} antagonists, suggesting that Ca^{2+} flux in plasma membrane is specifically required for transduction of the light stimulation.

Light-induced pigment migration of cultured melanophores is accompanied with the alteration in cell shape. When dispersed by illumination, melanophores, at first, expand in peripheral region of cytoplasm, and then pigments transport centrifugally. When aggregated in the dark, inversely, centripetal translocation of melanosomes is followed by the retraction of cytoplasm in the margin. Therefore, the cell shape of melanophores seems to be changed in accordance with every response to light. Ca^{2+} antagonists affect the light-induced alteration in cell shape of Oryzias melanophores. This fact suggests that Ca^{2+} flux in plasma membrane appears to be involved in light-induced changes in cell shape.

VIDEO AND ELECTRON MICROSCOPIC STUDIES ON CENTRIFUGAL AND CENTRIPETAL GRANULAR TRANSPORT IN DENDRITES OF GAMBUSIA MELANOPHORES
Naoshi Nakamura[1] and Masataka Obika[2]
Department of Physiology, Tokyo University of Fisheries, Tokyo[1] and Department of Biology, Keio University, Yokohama[2], Japan

Melanosome aggregation and dispersion in fish melanophores are generally accompanied by drastic changes in cell morphology. Whether these changes are produced passively by massive translocation of cytoplasmic constituents or they are actively engaged in the generation of the motive force for cellular responses remains to be settled. Video microscopy on isolated scale melanophores of the teleost Gambusia affinis indicates that the pigment granule transport toward the centripetal direction proceeds with a high velocity at the initial step of the response (5.8 ± 3.0 μm/sec), decreasing rapidly thereafter. In dispersion, on the other hand, pigment granules travel at a more or less uniform, but reduced rate (2.7 ± 1.8 μm/sec). The difference in the velocity may be due to the change in intracellular resistance during chromatophore responses or could be a reflection of the difference in the mechanism of force generation between dispersion and aggregation. The role of cytoplasmic microtubules that distribute in the cellular cortices of dendritic processes was examined through observations of the movement patterns of pigment granules and other cytoplasmic particles during the collapse and reformation of the dendrites.

ULTRASTRUCTURAL BASIS OF PHYSIOLOGICAL RESPONSES OF THE LEUCOPHORE-MELANOPHORE COMPLEX OF THE MEDAKA ORYZIAS LATIPES
Masataka Obika
Department of Biology, Keio University, Yokohama, Japan

Leucophores in the integument of the medaka are generally found in close association with overlying melanophores. Pigment cells in this teleost are under the control of adrenergic nerves, and melanophores that possess alpha adrenergic receptors respond to nervous stimulation with pigment aggregation (causes body lightening) while leucophores, which are predominantly controlled by beta adrenergic receptors respond to the same signal with pigment dispersal that also enhances lightening by increasing light reflectance. Thus, leucophore-melanophore complex represents an exquisite example of chromatophore unit in fish integument. Studies on the physiological responses of leucophores have been carried out mainly at light microscopic level, and the structural basis of their responsiveness has not yet been clarified.

Ultrastructural studies have shown that leucophores contain numerous small spherical vesicles that probably contain purine derivatives and rather poorly developed microtubule system. The present study deals with the role of microtubule and other cytoskeletal elements in the pigmentary responses. Innervation of adrenergic nerves into leucophore-melanophore complex, and the possible interaction between the two types of pigment cells are examined.

MECHANISM OF MOTILE IRIDOPHORES OF BLUE DAMSELFISH. N. Oshima, H. Kasukawa and R. Fujii. Department of Biology, Faculty of Science, Toho University, Funabashi, Chiba 274, Japan.

We found "motile" iridophores in the dermis of the blue damselfish, Chrysiptera cyanea. Their body hue changes very rapidly from characteristic cobalt-blue to dark violet or to greenish tone under certain conditions. As we reported in the last Conference in Giessen, such changes were primarily based on the activity of simple dermal chromatophore units, each of which is composed of several motile iridophores and a melanophore, and dominantly on the iridophore motility. In the present study, therefore, we intended to examine motile mechanisms of this unique light-reflecting chromatophore. Using split-fin preparations, the spectral peak reflected from the cells could be changed within the region from 380 nm to 530 nm in vitro in response to nervous stimuli and catecholamines. Probably, the distance between adjoining reflecting platelets changed simultaneously, leading to a shift of spectrum of reflected light, which may originate in the "non-ideal" thin-film interference phenomenon of the multilayered platelets. Furthermore, colchicine, vinblastine and podophyllotoxine inhibited the cell motility, though cytochalasin B had no effect. In addition, EHNA (erythro-9-[3-(2-hydroxynonyl)]adenine), a dynein ATPase inhibitor, blocked coloring response of the cells, suggesting an involvement of tubulin-dynein system in the damselfish iridophore motility.

CHROMATOPHORES AND COLOR CHANGE OF THE PHANTOM LARVA CHAOBORUS CRISTALLINUS. W.Weber, M.Grosmann, Univ. Cologne, Fed.Rep.Germany

The buoyancy of the phantom larva is achieved by 2 pairs of tracheal bladders, which are located in the thoracal and 7th abdominal segment. The surface of the bladder harbours dark chromatophores which display ameboid properties and shape changes which depend upon background illumination. The ultrastructural organisation and some functional aspects of the chromatophore system are presented.

CATECHOLAMINE-α-RECEPTOR COUPLING IN MELANOPHORES DOES NOT REQUIRE EXTRACELLULAR CALCIUM. Maria Aparecida Visconti and Ana Maria de Lauro Castrucci. Dep. Fisiologia Geral, Inst.Biociências, Univ. S.Paulo. São Paulo, Brasil, CP 11176.

In order to establish the appropriate experimental conditions to study the role of extracellular calcium on pigment migration, the influences of neuronal and extraneuronal uptake and of ß-adrenoceptor on melanosome aggregating response to catecholamines were investigated. Neither cocaine nor dexamethasone displaced the dose-response curves (DRC) to norepinephrine (NE) or phenylephrine (Phe). In the presence of propranolol (10^{-5}M) the DRC to NE was shifted about 5,8 times to the left, but not the DRC to Phe. The results suggested that the ß-adrenoceptor should be blocked in order to obtain the full aggregating response to NE. Therefore, the next experiments, in which the role of extracellular calcium in the catecholamine-α-adrenoceptor binding was studied, were performed in the presence of propranolol. In the absence of calcium, the DRC to Phe was not altered, but the DRC to NE was shifted about 3,5 times to the left. The results obtained with Phe suggest that calcium is not required for catecholamine-receptor binding. However, although we were not able to demonstrate the role of neuronal and/or extraneuronal uptake using the classical blockers, these processes are probably active in our preparation. Since both are dependent on extracellular calcium, they do not proceed in the absence of this cation and the amount of NE increased in the vicinity of the melanophores leading to a more sensitive assay to NE.

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STRUCTURAL AND FUNCTIONAL ASPECTS OF MOTILITY AND PIGMENT TRANSPORT IN CHROMATOPHORES OF THE SEA URCHIN CENTROSTEPHANUS LONGISPINUS W.Weber, M.Lehmann.Univ.Cologne; M.Hauser Univ. Bochum. Fed.Rep. Germany

From both indirect immunofluorescence (anti-Tubulin; anti-Actin) and from electronmicroscopic observations after cryofixation and -substitution, a conceptual model is presented for the dynamics of shape change and pigment translocation in chromatophores of the sea urchin. The prerequisite for light-dependent dispersion of pigment granules and the transition of cell shape from round to stellate is the microtubule system. In aggregated cells the microtubules are folded and appear as a condensed network of clearly discernible cables. The pigment granules are embedded within a structured continuum containing actin and other filaments of differing dimensions. ATP-dependent pigment dispersion is probably initiated within a filamentous network surrounding the perinuclear pigment-free cytocentral area. Upon contraction, the network may produce a hydraulic pressure which leads to centrifugal cytoplasmic/pigment streaming and a concomitant unfolding of the microtubule system. In contrast, cellular aggregation is ATP-independent and may be brought about by the release of stored energy.

THE EFFECTS OF α- AND β-MSH ON TYROSINASE ACTIVITY OF NORMAL HUMAN MELANOCYTES, CLOUDMAN S-91 MELANOMA CELLS AND HAIR PIGMENT IN YELLOW MICE, Leon M. Wilkins and Karla L. Stoner, Gillette Research Institute, Rockville, MD.

Commercially available forms of α- and β-MSH were compared for their in vitro and in vivo effects upon melanogenesis utilizing normal human melanocytes, Cloudman S-91 mouse melanoma cells, and yellow haired (A^y/a) mice . The superagonist ($Nleu^4$, $D\text{-}Phe^7$) α-MSH and synthetic porcine β-MSH caused similar elevations in tyrosinase activity of human melanocytes cultured in the absence of phorbol esters. Maximal elevation achieved was approximately two-fold at a dose of 10^{-8} M, and higher doses did not result in greater elevation of melanocyte tyrosinase activity. Cloudman S-91 melanoma cells showed a six-fold increase in tyrosinase activity after treatment with 10^{-8} M superagonist α-MSH, but only a three-fold increase in response to 10^{-8} M β-MSH. Response in human melanocytes was also found to vary, depending on source of the primary culture and baseline tyrosinase activity of that population of melanocytes. Human melanocyte populations with higher baseline tyrosinase activity did not respond to superagonist α-MSH. At equal dosages (2.5 μg/animal, 3 times/wk for 3 wks), superagonist α-MSH produced a shift in pigment production from pheomelanin (yellow) to eumelanin (black) pigment in previously plucked, regrowing hair of A^y/a mice, while β-MSH did not affect pigment production. Superagonist α-MSH was found to be 300^+ times as potent as native α-MSH in dose response studies on A^y/a mice.

Melanoma-Melanocyte Culture

The Invasive Behavior of Human Melanoma Cells May Be Related To A Gene Amplification Event. S.J. Bevacqua, C.W. Greeff and M.J.C. Hendrix, Departments of Molecular Biology, Microbiology and Immunology, and Anatomy, University of Arizona, Tucson, AZ.

A study was undertaken to determine whether human melanoma cells show cytological evidence of gene amplification after traversing amniotic basement membranes in vitro. A human melanoma cell line (A375P), which was shown to have exceptionally low metastatic capability in vivo (Koslowski, et al., JNCI, 72(4):913 1984) was used in this study. Cells were seeded onto $13cm^2$ sections of fresh human amnion denuded of epithelium ($3x10^6$ cells/section) using a modified Membrane Invasion Culture System chamber. Cells that were able to attach to and completely traverse the membrane within 12-16hr were collected from the chamber below the amniotic membranes and grown to 80-90% confluency. These cells were then examined for the presence of small, paired chromatin bodies known as double minutes (DMs), which are strongly associated with gene amplification events in mammalian cells. Using a standard staining procedure, DMs in cells that had passed through an amnion twice (A375P-2), or not at all (A375P), were quantitated within 15 doublings. Only 4% of the A375P cells contained 1-3 DMs/cell, whereas 24% of the A375P-2 cells contained 1-15 DMs/cell. An exceptional A375P-2 cell contained 25 DMs indicating that passage through the amniotic membrane causes a significant increase in the number of DMs. These data suggest that gene amplification may be an integral part of the mechanism by which human melanoma cells invade extracellular matrices during the process of metastasis.

EXPESSION OF DIFFERENTIATED FUNCTION IN CULTURED AVIAN MELANOCYTES. Raymond E. Boissy and Ruth Halaban, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Pure cultures of chicken melanocytes are obtained from neural tubes of 2.5 day old embryos. The tubes are placed in culture dishes, and melanoblasts migrate out. Proliferation of melanocytes is dependent on 12-O-tetradecanoyl-phorbol-13-acetate (TPA, 32 mM). The growth of contaminating cells is inhibited by medium low in calcium and magnesium. The melanocytes in culture are highly pigmented and have high tyrosinase activity. They synthesize ovoid premelanosomes with a lattice of melanofilaments. During maturation, the melanosomes become spherical and accumulate floccular deposits of melanin. Tyrosinase antiserum precipitates two proteins with molecular weights 68 and 82kD. These have a precursor/product relationship. Melanocytes cultured from mutant lines of chickens defective in melanin synthesis also express their defects in culture: Smyth chicken melanocytes express an increase in both tyrosinase activity and distribution prior to autophagocytosis and death; White Leghorn melanocytes are pigmented and have a short life span; tyrosinase-positive albino melanocytes have normal and abnormal premelanosomes, high tyrosinase activity, and a tyrosinase that is immunoprecipitable and has an aberrant intracellular localization; tyrosinase-negative albino melanocytes have ill defined premelanosomes and no immunoprecipitable tyrosinase or tyrosinase activity. These findings indicate that cultured melanocytes express their characteristic in situ phenotype in culture.

L-DOPA REGULATES PROLIFERATION OF MELANOMA CELLS. Jean Bolognia and John Pawelek, Department of Dermatology, Yale University School of Medicine, New Haven, CT.

It is well established that high concentrations of L-dopa (10^{-4}-10^{-3}M) are toxic to pigmented cells. In this study we examined the effects of lower concentrations of L-dopa on the growth of a variant line of the Cloudman S91 mouse melanoma, designated "cA^{dep}." In order to proliferate, cells from the cA^{dep} line have an absolute dependence on MSH or other agents which raise cAMP levels. Cells were cultured in Ham's F10 medium. Additions were MSH, isobutylmethylxanthine (IBMX), or L-dopa. MSH stimulates growth of cA^{dep} cells through a stimulation of the adenylate cyclase system. IBMX stimulates the cells by preventing break-down of cAMP. Results were as follows: L-dopa, 10^{-4}M, was cytotoxic causing cell lysis and a decreased cell number compared to control cultures in plain medium. L-dopa, 10^{-5}M, was not cytotoxic and did not change the number of cells compared to control cultures. Likewise, L-dopa, 10^{-5}M, had little or no effect on the stimulatory action of IBMX on the cells. Surprisingly, however, L-dopa, 10^{-5}M, completely inhibited the stimulatory action of MSH on cellular proliferation, but had no effect on the stimulatory action of MSH on melanogenesis. We conclude that L-dopa has a specific effect on the MSH-receptor complex, and/or the adenylate cyclase system which modifies proliferation of the cA^{dep} cell line. The findings open the possibility of a new role for L-dopa as a growth regulator of melanoma cells.

NEUTRAL MELANOMA TRANSFORMING GROWTH FACTOR (NMSA) A NOVEL HUMAN PLATELET PROTEIN

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We have isolated a unique acid pH sensitive melanoma transforming growth factor activity from human platelets. The growth factor contains disulfide links and runs with a Mr 60,000 on a tsk 3000SW HPLC column. NMSA promotes and induces the clonogenic growth of human melanoma cells in soft agar. All known platelet associated growth factors (TGF-beta and PDGF), insulin, and epidermal growth factor do not induce the clonogenic growth of human melanoma cells in soft agar.

NMSA is mitogenic to human melanoma cells and aortic endothelial cells, but does not promote the growth of human melanocytes. We hypothesize that NMSA normally acts as an endothelial growth factor and that expression of the NMSA receptor may be associated with the transformation process for human melanocytes.

THIN-SECTION ELECTRON MICROSCOPY AND FREEZE-FRACTURE REPLICATION OF PURE CULTURES OF MELANOCYTES DERIVED FROM NORMAL HUMAN FORESKIN EPIDERMIS. A.S. Breathnach, E.J. Robins, Y. Bhasin, L. Ethridge, C. Patzold, D. Bennett. Department of Anatomy, St.Mary's Hospital Medical School, London, UK.

Pure cultures of melanocytes were obtained from normal human neonatal foreskin by a modification of the method of Eisinger and Marko, using TPA and cholera toxin. The pictures tell the story, but attention may be drawn to the following features seen in cultures harvested at passage 26 and containing up to 5.4×10^4 cells/ml:

1) There was no evidence of extrusion of melanosomes into the medium, and many cells presented a ''constipated'' appearance. 2) There was considerable variation in melanosome morphology, and aggregates of different types were present within many cells. 3) Specialized contacts of simple desmosomal type were occasionally seen on cell membranes of apposed cells. 4) On replicas of plasma membrane, particle density on the P-face is 790 ± 82, and on the E-face 215 ± 51, with 60% lying in the range of 7-10 nm in diameter. These figures are within the range of MAP particle density and diameter of cells in general.

CONTROL OF NEL-M1 HUMAN MELANOMA CELL GROWTH AND PROTEIN SECRETION BY TRIAMCINOLONE ACETONIDE. Dennis M. DiSorbo, Bernice M. Martin, Arlene P. Fabian, and Carl Franzblau. Section of Medical Oncology, Evans Department of Medicine, and Department of Biochemistry, Boston University Medical Center, Boston MA 02118.

The human melanoma cell line, NEL-M1, is growth inhibited when exposed to the synthetic glucocorticoid, triamcinolone acetonide (TA). To further study the direct effects of TA on these cells, NEL-M1 cells have been established to grow in Ham's F12 medium in the absence of serum, hormones, and exogenous growth factors. Initial experiments show that cells cultured in 10 nM TA for 24 and 48 h had a 16 and 34% reduction in (^{3}H) thymidine incorporation, respectively, compared to controls. After a 96 h exposure to 10 nM TA, cells began to round up and detach from the substratum. If the detached cells were cultured in fresh medium, they attached to the substratum, suggesting that TA did not induce its effect through cytotoxicity. When the medium proteins from the 96 h cultures were analyzed on SDS polyacrylamide gels, two major differences were observed between controls and the TA treated cultures. TA treatment resulted in the appearance of a new protein in the 100k dalton range and the disappearance of a protein in the range of 50k daltons. These results suggest that one mechanism by which glucocorticoids regulate the growth and attachment of NEL-M1 cells is through the modification of the synthesis and/or secretion of extracellular proteins. Supported by grant GRS NIH RR05487-23.

FIBROBLAST GROWTH FACTOR IN THE PRESENCE OF dbcAMP IS MITOGENIC TO HUMAN MELANOCYTES. Ruth Halaban, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

The factors required for the proliferation of normal melanocytes in culture may be key components for understanding of the processes involved in transformation and progression to metastatic melanomas. The putative melanocyte growth factor elaborated by several cell lines and tissues (1-3), requires the presence of stimulators of intracellular levels of cyclic adenosine monophosphate (cAMP). Because tissues in which melanocyte mitogen is abundant are rich in fibroblast growth factor (FGF), we tested whether partially purified FGF (Biomedical Technologies, Inc.) was mitogenic to melanocytes. Our data show that FGF (100 ng/ml) together with dbcAMP (1 mM), supports the proliferation of neonatal human melanocytes. An antigenically related growth factor, endothelial mitogen (EM, partially purified, Biomedical Technologies, Inc.) was as active as FGF. FGF, EM, or dbcAMP alone had no mitogenic activity. It is thus possible that the melanocyte growth factor of tissue extracts is related to the FGF-family and that among the processes involved in the progression to metastatic melanomas is production of FGF-like substances, constitutive activation of receptors independently of the ligand, and acquisition of independence from exogenous cAMP.

1. Eisinger M, Marko O, Ogata S-I, Old LJ. Science 229:984, 1985.
2. Wilkins L et al. J Cell Physiol 122:350, 1985.
3. Halaban R et al. JID, in press 1986.

PHOSPHORYLATED ISOMERS OF L-DOPA STIMULATE MSH BINDING ACTIVITY AND RESPONSIVENESS TO MSH IN CULTURED MELANOMA CELLS. John McLane, Marilyn Murray, Michael Osber, and John Pawelek, Department of Dermatology, Yale University School of Medicine, New Haven, CT.

At high concentrations (1mM) phosphorylated isomers of L-dopa are taken up by melanoma cells and converted to L-dopa and inorganic phosphate by cellular phosphatases. The L-dopa is then converted into melanin by tyrosinase and the phosphate is incorporated into acid-precipitable material--presumably nucleic acids and proteins (Pawelek and Murray, Cancer Res. 46:493,1986). We investigated the effects of lower concentrations of phosphodopas (.001 - .01mM) on cultured Cloudman melanoma cells. Using ^{125}I-labelled β-MSH as a tracer, we found that preincubation of melanoma cells with these low concentrations of phosphodopas resulted in a 3-4 fold stimulation of MSH binding capacity by the cells. Scatchard analyses indicated that the stimulation of receptor activity occurred through an increase in the affinity of the receptors for MSH rather than through an increase in the number of receptors. The increased receptor activity was accompanied by an increased responsiveness of cells to MSH. Cells treated with phosphodopas (0.001 - 0.01mM) for 48 hours showed a 3-4 fold increase in both tyrosinase activity and melanin content when exposed to MSH. Under these same conditions phosphodopa treatment without exposure to MSH had little or no effect on the cells. We conclude that apparently phosphodopas can act either directly or indirectly as co-factors for MSH receptors.

GROWTH-RELATED PROTEINS IN NORMAL HUMAN MELANOCYTES AND IN MELANOMAS. Ruth Halaban, Sikha Ghosh, and Francis D. Alfano, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Proteins that are induced, suppressed, or activated by factors that stimulate the proliferation of normal human melanocytes in culture may be involved in the regulation of pigment cell proliferation. Using extracts of metabolically labeled melanocytes and one-and two-dimensional gel electrophoresis, we found an increase in abundance of four proteins (p53, p38, p37, and p31), and a decrease in two others (p35 and p32), in response to agents that stimulate melanocyte proliferation, i.e., TPA, placental extract, and IBMX. In the two human melanoma cell lines tested, p53, p38, and p37 were constitutively expressed, and p35 was constitutively suppressed. The p53 of pigment cells has an acidic isoelectric point and is neither the known p53 induced by SV40, nor is it ornithine decarboxylase.

Our studies have also shown that in normal melanocytes TPA (12-O-tetradecanoyl-phorbol-13-acetate) induces the phosphorylation of two proteins with apparent molecular weights of 28,000 and 77,000 and decreases the levels of protein kinase C activity. Further identification of the growth-related proteins in normal human melanocytes and in melanomas may help us understand the mechanism of malignant transformation.

PHOSPHORYLATION OF A 90kd PROTEIN IN CLOUDMAN MELANOMA CELLS CORRELATES WITH RESPONSIVENESS TO INSULIN, EXPRESSION OF HIGH-AFFINITY BINDING SITES FOR INSULIN, AND APPARENT PROTEOLYSIS OF THE INSULIN RECEPTOR. Tessie McNeely, Andrzej Słominski, and John Pawelek, Department of Dermatology, Yale University School of Medicine, New Haven, CT.

A phosphoprotein of 90k daltons ("pp90") appears to be involved in the growth response of Cloudman melanoma cells to insulin. Discovery of pp90 resulted from comparisons of insulin-responsive lines to 3 non-responsive variants. The variants were unable to phosphorylate pp90 in vitro. A survey of 24 Cloudman lines showed that phosphorylation of pp90 correlated strongly with the generation time of each of the lines in insulin. In addition, Scatchard analyses of insulin-resistant variants showed greatly reduced ability to express high affinity receptors for insulin. Analyses of insulin-dependent phosphorylation of the β-subunit of the insulin receptor in vitro revealed that whereas wild-type lines displayed a single β-subunit of 95k daltons, variant lines displayed two phosphoproteins of 95 and 85k daltons respectively. Bovine tyrpsin inhibitor did not influence phosphorylation or mobility of the wild-type β-subunit but prevented the appearance of the 85k dalton band in a variant line. The results indicate that insulin-resistant cells display β-subunits which are sensitive to proteolysis, and that this correlates with cellular response to insulin, and the ability to phosphorylate pp90. A unifying explanation for these results could be that phosphorylation of pp90 affects proteolysis of the insulin receptor.

RAPIDLY DIVIDING NORMAL HUMAN MELANOCYTES EXPRESS IN CULTURE MELANOMA-ASSOCIATED ANTIGENS. M.L. Mancianti, U. Rodeck, J. Jambrosic, and M. Herlyn. The Wistar Institute, Phila., PA 19104.

Melanocytes from newborn foreskin grew rapidly in culture (up to 1.0 doubling per day) when maintained in MCDB 153 medium supplemented with calcium, insulin, transferrin, epidermal growth factor, bovine pituitary extract, and 2% fetal bovine serum. In contrast to resting melanocytes from normal skin, cultured melanocytes expressed most major melanoma-associated antigens tested, e.g., NGF-receptor, proteoglycan, transferrin related p97 antigen, p120 Kd, and gangliosides 9-O-acetyl GD_3 and GD_3. HLA-DR antigen and ganglioside GD_2 were not expressed or only at very low levels. Most melanocyte cultures, including clones and melanocytes sorted by rosetting with monoclonal anti-NGF receptor antibody, lost after several subpassages their characteristic bipolar morphology and they also lost the expression of NGF-receptor and p97 antigen. On the other hand, a minority of melanocyte cultures maintained their characteristic bipolar to spindle morphology. These cells retained the expression of all melanoma associated antigens and even gained expression of HLA-DR antigen. Our studies indicate that cultured rapidly dividing melanocytes undergo antigenic changes associated with malignancy.

PARTIAL PURIFICATION AND CHARACTERIZATION OF MELANOCYTE GROWTH FACTOR FROM A MELANOMA CELL LINE (McGF-M). S. Ogata, Y. Furuhashi, M. Eisinger. Memorial Sloan-Kettering Cancer Center, New York, N.Y.

The extracts of 3 different cell types including melanoma cells have recently been found to contain potent mitogenic factors which stimulate melanocytes to grow in vitro (1). We report here, partial purification and characterization of a melanocyte growth factor derived from a human melanoma cell line SK-MEL-178. The activity was extracted from melanoma cells as previously described (1) and monitored by incorporation of ^{3}H-thymidine in normal human melanocytes. The extract was concentrated by ammonium sulfate precipitation and fractionated by 2-step gel filtration chromatography on Sephadex G-100, ion exchange chromatography on DEAE and affinity chromatography on heparin-Sepharose. McGF-M is heat labile (100°C,5 min), acid (pH 3) and alkaline (pH 10) resistant, trypsin sensitive and 2-mercaptoethanol insensitive. The apparent molecular weight of the factor is 13,000 dalton by gel filtration and SDS-polyacrylamide gel electrophoresis.

Because of the affinity of McGF-M to immobilized heparin, possible relationship with fibroblast growth factors was investigated. Based on different mitogenic activities of McGF-M and FGFs for human melanocytes and NIH3T3 fibroblasts, together with the differences in some biochemical properties, they are clearly distinguishable.

1. Eisinger M, et al. Science 229:984,1985.

CELL CYCLE SPECIFICITY OF MSH AND RETINOIC ACID INDUCED GROWTH INHIBITION OF B16 MOUSE MELANOMA. R.M. Niles, and B.P. Loewy, Boston Univ. Sch. of Med. Boston, MA. 02118

Through the use of flow microfluorimetry we have found that both MSH and retinoic acid (RA) arrest B16 cells in the G1 phase of the cell cycle. When these agents were removed from the arrested cells, DNA synthesis was initiated within 2h. This indicates that both MSH and RA block cell cycle progression late in G1. RA-treated cells had a 50% reduction in protein synthesis, however total protein content/cell was the same as untreated cultures. Pulse-chase studies with ^{3}H-leucine demonstrated that the turnover of total protein was markedly suppressed in RA-treated cells. These changes were not observed in MSH-treated cells. Tyrosinase activity was increased by MSH and it's activity remained elevated at least 18h after the hormone was removed. An inhibitor of tyrosinase activity was found to co-induced by MSH. The inhibitor activity was also high in confluent cultures where enhanced melanin production was observed. In RA-treated cells there was no change in tyrosinase activity, however when RA was removed from the culture, tyrosinase activity declined dramatically within 6h. These results indicate that although both RA and MSH block B16 melanoma cells at about the same point in G1, the mechanism(s) by which this is achieved is probably different for each agent. Supported in part by grant CA32543 from NCI

REPETITIVE PASSAGE OF B16-F10 CELLS THROUGH HUMAN AMNIONS PRODUCES AMELANOTIC MURINE LUNG NODULES: A COMPARATIVE IN VITRO AND IN VIVO STUDY. Bruce Persky, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois.

Human amelanotic melanoma is difficult to diagnose, presents with an unknown primary site one-third of the time, and often is clinically aggressive. It is of interest to test the hypothesis that amelanotic phenotype is related to tumorigenic potential. In this study B16-F10 melanoma cells were collected after a single passage through a human amnion and grown in tissue culture. The cells were replated onto a second amnion. The cycle of plating cells, invasion, and subsequent growth in tissue culture of the invading subpopulation was repeated five times. The in vitro invasion rate for B16-F10 cells remained essentially unchanged for the six passages. Tail vein injections into C57BL6 mice of $1x10^5$ B16-F10 cells from the first amniotic passage resulted in an average of 29+12 discrete, melanotic lung tumors per animal (n=7) whereas injection of passage six cells resulted in 300+ amelanotic and melanotic lung tumors per animal (n=11). The in vivo lung data were interpreted as showing that amnions selected a more tumorigenic subpopulation of cells and that there was a concomitant phenotypic change of lung nodules from melanotic to amelanotic and melanotic. Interestingly, the selection of a more tumorigenic cell subpopulation in vivo was not correlated to increased invasion rates in vitro. "Supported by Grant 887-12 from the Bane Charitable Trust and by BRSG Grant 447-31 from USPH."

IN VITRO GROWTH CHARACTERISTICS OF MELANOCYTES FROM NORMAL AND VITILIGO SUBJECTS. Neelu Puri, Abburi Ramaiah and Manoj Mojamdar, Dept. of Biochemistry, All India Inst. of Medical Sciences, New Delhi-110029, India.

The in vitro growth characeristics of melanocytes from uninvolved and perilesional skins of vitiligo subjects have been investigated in comparison to those from normal donors. Normal melanocytes have been found to grow exponentially in the presence of 10^{-11}M cholera toxin and 10ng/ml of TPA in routine media. They could be trypsinized upto 3-4 passages. Melanocytes of the uninvolved skins of vitiligo subjects manifested a lag of 8-10 days for the onset of growth and they could not be passaged. Melanocytes of the perilesional skins failed to grow under these conditions. Only in a few cases where they were normally pigmented the melanocytes grew after a lag of 15 days. The initial seeding capacity of the melanocytes was 50 and 25 percent respectively for uninvolved and perilesional skins as compared to normal skins. Fetal lung fibroblast extracts that promoted the growth of normal adult melanocytes also enhanced the growth rate of melanocytes of the uninvolved skins without affecting the lag. Perilesional skin melanocytes exhibited a burst of growth in the presence of these extracts, 10-11 days after seeding. Significantly, the melanocytes of the uninvolved skins of vitiligo subjects could be passaged 3-4 times in the presence of these extracts. Our findings indicate that melanocytes themselves from vitiligo patients are defective.

CHANGES IN BIOCHEMICAL PROFILES OF THE CYTOSKELETAL PROTEINS BEFORE AND AFTER MELANOMA CELL INVASION; Richard E.B. Seftor, Mary J.C. Hendrix and Anne E. Cress, Depts. of Anatomy and Radiation Oncology, College of Medicine, Univ. of Arizona, Tucson, AZ.

An important consideration in the invasive movement of melanoma cells is the role of cytoskeletal proteins during the dynamic process of metastasis. One of the major steps of invasion involves tumor cell locomotion into regions of tissues modified by cell secreted enzymes. The composition and architecture of the cytoskeletal proteins are directly associated with the ability of tumor cells to invasively migrate and disseminate through the body. In order to understand the mechanisms of tumor cell motility, we undertook a biochemical analysis of possible changes in the cytoskeletal proteins of high and low metastatic variants (A375M and A375P, respectively) of a human melanoma cell line before and after invasion of an extra cellular basement membrane in vitro. Non-equilibrium pH gradient gel electrophoresis (NEPHGE) was run in the first dimension (basic proteins are better resolved by this method than by conventional isoelectric focusing) and standard SDS-PAGE run in the second dimension. Results indicate a qualitative difference in the proteins from detergent extracted cells (i.e. preparations enriched in the cytoskeletal proteins) of the two metastatic variants and could, therefore, directly relate to the metastatic potential of the cell line(s) under consideration. Hopefully, this investigation will elucidate the role of specific cytoskeletal proteins responsible for rapid motility through tissues. NIH 1R01 CA42475

INHIBITORY EFFECTS OF AZELAIC ACID AND OTHER DICARBOXYLIC ACIDS ON GROWTH AND MELANOGENESIS OF HARDING-PASSEY MELANOMA (HPM-73) CELLS IN DEFINED SERUM-FREE CULTURE MEDIA.

D.O. Schachtschabel, D. Thome, B. Salzer, Institut für Physiologische Chemie, Philipps-Universität, Lahnberge, Marburg, FRG.

Exponentially growing HPM-73 monolayer cells in defined, serum-free culture media (Schachtschabel et al., Pigment Cell 1985, 515-519) were treated (up to 10 days) with undecandioic acid (C_{11}), sebacic acid (C_{10}), azelaic acid (C_9), suberic acid (C_8), pimelic acid (C_7), adipic acid (C_6), or glutaric acid (C_5) in concentrations between 2.5×10^{-3} and 1×10^{-2} M. Significant inhibition of cell proliferation and melanin formation occurred with C_8- C_{11} dicarboxylic acids (the inhibitory effects were increasing with increasing chain length), while C_5- C_7 dicarboxylic acids were nearly ineffective. The inhibitory effects were reduced by the addition of serum (10 % FCS) to the culture media. Growth cessation was accompanied by the occurrence of cells with slim, often bipolar morphology, while control cells appeared larger and more randomly oriented.

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IN SITU MELANIN ASSAY FOR MELANOMA CELLS IN CULTURE. W. Siegrist, A.N. Eberle, S. Stutz and J. Girard Department of Research, University Hospital and University Children's Hospital, CH-4031 Basel, Switzerland.

Structure-activity studies with large numbers of peptides inducing melanogenesis in cultured melanoma cells require a rapid melanin assay. Since extraction of melanin following hormone stimulation is cumbersome, we have developed an in situ melanin assay which is based on densitometric measurement of total melanin formed by the cells in a certain time period. Briefly, B-16 mouse melanoma cells were plated on 96-well Costar trays and incubated with hormone for three days. The total melanin formed in each well was determined directly with an automatic Elisa reader at a wavelength of 405 nm. Calibration of the instrument with a dilution series of synthetic melanin showed a linear relationship between optical density and melanin content within the concentration range required for the assay. Structure-activity studies with more than 20 MSH peptides showed that the assay was reliable and very sensitive. The ED_{50} for α-MSH was 1.2×10^{-11} M, for [Nle^4, D-Phe^7]-α-MSH 1×10^{-12} M and for ACTH(1-24) 2.7×10^{-10} M. Thus, this in situ assay is about 2-10x more sensitive than the tyrosinase assay and hence the most sensitive melanoma cell assay for MSH peptides available to date. The principle of the assay could also be applied to other types of melanoma cells, such as Cloudman S91, with slightly modified conditions.

A METHOD FOR CULTURING OF NORMAL MURINE MELANOCYTES. A. Tamura, R. Halaban, and A. B. Lerner. Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Murine melanocytes in culture can provide genetically different cell lines for the study of problems in pigmentation because several inbred strains of mice carrying relevant mutations are available. Advances in culture methods for human melanocytes helped us devise optimal conditions for the growth of murine melanocytes. We found that the dermis from 1 - 3 day-old newborn mice is an excellent source for melanocytes. Our method is as follows: A mouse is anesthetized with pentobarbital, and the skin is peeled from the dorsum and incubated with trypsin (0.25%, 1 hr at 37° C). The dermis is then sliced into small pieces and gently triturated. The dissociated cells are seeded into a 25 cm^2 flask in Ham's F-10 medium supplemented with 8% Nu-serum, 8% newborn calf serum, 10 ng/ml 12-O-tetradecanoyl-phorbol-13-acetate (TPA), 0.1 mM isobutylmethyl xanthine (IBMX), and 50 g/ml human placental extract (melanocyte medium). After 2 days, the cultures are incubated with melanocyte medium supplemented further with 80 g/ml geneticin (G418) for 2 - 3 days to kill contaminating fibroblasts. We tested several culture conditions and found that all factors, TPA, IBMX, and placental extract, were needed for optimal growth. Dibutyryladenosine cyclic monophosphate (0.1 mM) could replace IBMX. Using this technique we were successful in growing melanocytes in culture from C57BL/6J and vitiligo (C57BL/6J Ler-vit/vit) mice.

Clinical Melanoma

CELL KINETICS AND VIABILITY OF A HUMAN MELANOMA CELL LINE EXPOSED TO DICARBOXYLIC ACIDS IN TISSUE CULTURE. A.S. Breathnach, E.J. Robins, Y. Bhasin, L. Ethridge, M. Nazzaro-Porro,[1] S. Passi,[1] and M. Picardo[1]. Department of Anatomy, St. Mary's Hospital Medical School, London, and Istituto Dermatologica S. Gallicano, Rome[1].

Cultures of human melanoma cell line B0008 were exposed to the disodium salts of azelaic acid (C_9 2Na), adipic acid (C_6 2Na), dodecanedioic acid (C_{12} 2Na) at 10^{-2}M and 5 X 10^{-2}M for 24 hrs. None of the diacid salts had a significant effect on growth rate or viability of the cells, at 10^{-2}M for 24 hrs. nor had C_6 2Na any effect at 5 X 10^{-2}M. At 5 X 10^{-2}M for 24 hrs, both C_9 2Na, and C_{12} 2Na had a significant effect in reducing both growth and viability. These effects were accompanied by morphological evidence of cell death, and swelling of mitochondria and accumulation of lipid droplets within cytoplasm of still viable cells was seen by thin section transmission electron microscopy.

The results parallel previous observations on growth kinetics and morphology of murine (Harding-Passey and Cloudman) melanoma cells exposed to higher concentrations (10^{-1}M) of dicarboxylic acids for shorter periods (1-6 hrs), and confirm the conclusion that the mitochondrion is a prime target for their biological and toxic effect.

4-OHA AND THE CELL CYCLE

D.L.Dewey and J.L.Holden. Cancer Research Campaign, Gray Laboratory, Mountvernon Hospital, Northwood, Middlesex, England.

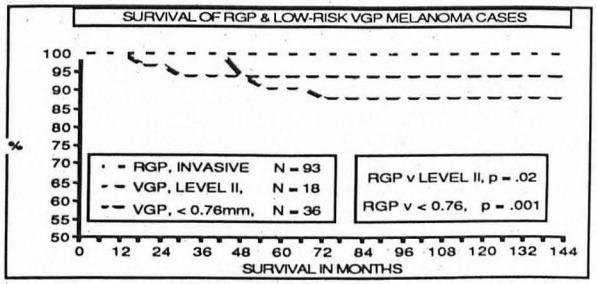
4-OHA is used in the treatment of recurrent malignant melanoma (1). It is selective for melanocytes in that only they contain tyrosinase which, among other things, converts 4-OHA into the toxic anisole orthoquinone (2). 4-OHA also has another effect on cells which is not selective for melanocytes but for dividing cells in general. This effect mimics that of the chemotherapeutic agent hydroxyurea. It is not yet known what proportions of the two effects are responsible for the effectiveness of the drug in clinical use.

The present demonstration attempts to quantify these two aspects of 4-OHA in vivo and in vitro using melanoma cells in culture and animal tumour models.

(1) B.D.G.Morgan (1984) In Hydroxyanisole Ed P.A.Riley.IRL press.
(2) J. L. Holden et.al.(1984) In Hydroxyanisole. Ed P. A. Riley. IRL press.

EARLY (RADIAL GROWTH PHASE, RGP) MALIGNANT MELANOMAS ARE BIOLOGICALLY BENIGN AND SHARE IMMUNOHISTOCHEMICAL MARKERS WITH NEVI. Elder DE, Guerry D, Clark WH Jr, Stewart R, Van Horn M, Herlyn M. The Pigmented Lesion Group, University of Pennsylvania, Philadelphia, Pa.

Microstaging of melanoma (MM) identifies "low-risk" subsets, but a few low risk lesions metastasize. Among 302 invasive MM with 7 year followup, survival of patients with 98 level II MM and 117 "thin" MM (< 0.76 mm) was 98% and 97%. "Plaque stage" invasive RGP MM lack tumorigenic growth (vertical growth phase, VGP); their survival is 100%, different from level II and "thin" lesions with VGP (94 and 88%, chart)



In frozen sections, we stained 2-7 examples of nevi, RGP and VGP primary MM, and metastases with fifteen monoclonal antibodies, identifying three groups of "Pigmented Lesion Antigens" (PLA):

Melanocytes.....PLA I⁻ II⁻ III⁻ Nevi & RGP.....I⁻ II⁻ III⁺
VGP.....PLA I⁻ II⁺ III⁺ Metastases.....I⁺ II⁺ III⁺

RGP MM, a clinically indolent and histologically "microinvasive" lesion, lacks tumorigenic growth (VGP), does not metastasise, shares antigens with nevi, and is therefore not a fully-evolved malignancy.

AN IN VITRO CORRELATION OF IN VIVO TUMOR METASTASIS. Hendrix, M.J.C., Seftor, E.A., Gehlsen, K.R., and Misiorowski, R.L., Departments of Anatomy and Surgery, College of Medicine, University of Arizona, Tucson, AZ.

An in vitro study was undertaken to examine the possible correlation between invasion profiles measured in vitro and in vivo lung metastases produced in the nude mouse by high and low metastatic varients of a human melanoma cell line (A375). A high metastatic varient (A375M) was previously established by cloning the lung tumor nodules produced by i.v. injection of the parental line (A375P) in young BALB/c nude mice (Kozlowski et al, JNCI, 72(4):913, 1984). The metastatic heterogeneity of these two varients were tested in vitro in our lab in the Membrane Invasion Culture System (MICS), which uses the human amnion assay (Liotta et al, Cancer Lett. 11:141, 1980). For this assay the A375M and A375P lines were split and then labelled with ^{14}C-Thymidine (0.25uCi/ml in DMEM with 2% FBS) for 72 hr. 5 x 10^4 cells were seeded in individual wells in MICS chambers containing fresh human amnions denuded of epithelium. 3 days in vitro, the A375M line achieved an invasion profile of 9.3% cells which had completely traversed the amniotic membranes compared with 3.4% invasion by the A375P line ($p<0.001$). In addition, plasminogen activator (PA) activity was measured for both lines, and A375M cells produced >5 fold more PA than the lower metastatic A375P cells. These data suggest that the in vitro invasion profiles produced by the A375M and A375P lines. are reflective of their metastatic capacity in vivo. Also, the amount of PA produced by the two varients correlates with their degradative capabilities.

EFFECTS OF α-MELANOTROPINS ON MELANOMA TUMOR CELL INVASION AND METASTASIS. Gehlsen, K.R., Hendrix, M.J.C., Hadley, M.E., and Levine, N. Department of Anatomy and Section of Dermatology, College of Medicine, University of Arizona, Tucson, Az. 85724.

The effects of α-Melanocyte Stimulating Hormone (MSH)$_4$ and a superpotent analogue of MSH ([Nle^4-D-Phe^7]- α-MSH) on in vitro tumor cell invasion and on in vivo lung metastasis formation was studied. In vitro invasion assays using Cloudman S-91 murine melanoma cells were performed with the Membrane Invasion Culture System (MICS) in the presence or absence of 10^{-7}M MSH or the analogue. These data demonstrate no significant effect of either hormone on tumor cell invasion in vitro. In vivo experiments using the DBA/2J syngeneic host were performed in concert with the in vitro investigations to assess the effects of the melanotropins on primary tumor growth and metastasis formation. Mice were injected either via the lateral tail vein (for experimental metastasis assay) or intracutaneously (for spontaneous metastasis assay) with S-91 melanoma cells and then treated via intraperitoneal (I.P.) injection of either the melanotropins in a final concentration of 10^{-3}M in 0.2cc or with normal saline (controls). The results suggest an enhancing effect of the melanotropins on survival and proliferation of tumor cells localized in the epidermis or the lungs but no effect on the metatatic process. This work was supported in part by a Cancer Biology Training Grant CA09213 (KRG).

A STUDY OF THE CYTOTOXIC ACTIONS OF 4-OHA AND ITS HUMAN PHARMACOKINETICS

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The toxicity of the phenolic depigmenting agent 4-hydroxyanisole (4-OHA) has been studied in vitro using pigmented and unpigmented Harding-Passey cells. Pigmented cells were a factor of ten more sensitive to 4-OHA than were the unpigmented varients. When 4-OHA was oxidised by mushroom tyrosinase cellular toxicity correlated with the production of 3, 4-anisylquinone which binds to the cell membrane. The relationship between this binding and cellular toxicity was investigated.

Pharmacokinetic data is presented from patients receiving 4-OHA therapy for malignant melanoma. The drug was cleared rapidly with a half-life of 9 minutes. Intra-arterial infusion of 6.7 mMoles/hr for 4 days resulted in plasma concentrations ranging from 0.01 to 0.3mM.

MECHANISM OF SELECTIVE TOXICITY OF 4-S-CYSTEINYL-PHENOL AND 4-S-CYSTEAMINYLPHENOL TO MELANOMA CELLS.
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Our previous studies showed that 4-S-cysteinyl-phenol (4-S-CP) and 4-S-cysteaminylphenol (4-S-CAP) inhibit the growth of malignant melanoma and that [^{3}H]4-S-CP is selectively accumulated in melanoma cells. In this study we examined kinetic constants of CP and CAP as substrates for tyrosinases and their properties as sulfhydryl scavengers. 4-S-CP and 4-S-CAP were found to be much better substrates for mush-room tyrosinase than L-tyrosine while their 2-S isomers were not the substrates. 4-S-CP and 4-S-CAP were also good substrates for mammalian tyrosinase. Upon tyrosinase oxidation the two phenols conjugated with cysteine to form the cysteinyl derivatives of the corresponding catechols in high yields. In addition, the tyrosinase oxidation product of 4-S-CP had a poor ability to conjugate with alcohol dehydrogenase, a sulfhydryl enzyme, while that of 4-S-CAP had a much higher ability; the finding parallels the fact that 4-S-CAP is much more potent in melanoma growth inhibition than 4-S-CP. These results suggest that in melanoma cells these phenols are oxidized by tyrosinase to the corresponding o-quinone forms, some of which conjugate with proteins through cysteinyl residues, thus exerting cytotoxic effects.

SELECTIVE MELANOCYTOTOXICITY OF 4-S-CYSTEAMINYLPHENOL AS JUDGED BY DEPIGMENTATION OF BLACK HAIR FOLLICLES IN MICE:
Yoshiko Ito and Kowichi Jimbow, Department of Dermatology, Sapporo Medical College, Sapporo, Japan.

In our previous reports (Miura et al. J Invest Dermatol 84:320, 1985, Jimbow et al. J Invest Dermatol 84:355, 1985), we have shown that 4-S-cysteinylphenol (4-S-CP) and 4-S-cysteaminylphenol (4-S-CAP) possess some anti-melanoma effect in vivo. This study evaluates the depigmenting potency of 4-S-CAP by black hair follicles, and clarifies the mechanism of toxicity by electron microscopy. 4-S-CAP was injected s.c. on the black skin of C57BL mice (3-day-old and 8-week-old adult mice). We found (a) that 4-S-CAP causes the depigmentation of black hair in both new-born and adult mice, (b) that depigmentation is manifested by a decrease in synthesis as well as transfer of melanosomes and degeneration of melanocytes with the swollen membranous organelles, (c) that no degenerative changes are seen in keratinocytes of black hair follicles, (d) that the melanocytes of albino hair follicles are not affected by 4-S-CAP even though they possess numerous melanosomes with unmelanized stages, and (e) that depigmentation appear to be most significant in the hair follicles of early anagen phase. These findings indicate the selective melanocytotoxicity of 4-S-CAP which is mediated through the presence of active tyrosinase. 4-S-CAP appears to provide a new modality for treating hypermelanotic lesions in the skin.

TREATMENT OF DISSEMINATED B16 MELANOMA BY MELPHALAN, CIS-PLATINUM AND CYCLOPHOSPHAMIDE.
Kanclerz, A. and Chapman, J.D. Radiobiology, Cross Cancer Institute, Edmonton, Canada T6G 1Z2

Most chemotherapeutic agents have been selected for antitumor activity against injected tumor cells (e.g., leukemic) or against the primary of selected animal tumors. We have measured the efficacy of currently used cytotoxic drugs against disseminated disease and spontaneous metastases of B16 melanoma (wild type) and of Lewis lung carcinoma. Metastatic disease was treated after the primary tumors had been amputated. Melphalan (10 mg/kg i.p. on day 1 and 5 after tumor surgery) enhanced the growth of pulmonary metastases of both tumors as measured by number, range and incidence. An increase of amelanotic secondary foci was observed with B16 melanoma. Cis-platinum (6 mg/kg i.p. on day 1 after tumor surgery) was found to suppress the dissemination of both tumors, the drug being more effective in fractionated treatment schedules (2.4 mg/kg i.p. on days 1 through 5 after tumor surgery). Its action appeared to be larger against amelanotic than against melanotic metastases arising from "wild type" B16 melanoma. Cyclophosphamide (200 mg/kg i.p. on day 1 after tumor surgery) was found to have little or no effect on the spread of either tumor. These data indicate that disseminated animal tumor cells can be resistant and possibly refractory to the cytotoxic action of some chemotherapeutic drugs and that melphalan treatments can actually promote the growth of metastatic disease.

A PHASE I STUDY OF MONOCLONAL ANTIBODY THERAPY IN DISSEMINATED MELANOMA. A.E. Lichtin, D. Guerry, D.E. Elder, R. Hamilton, D. LaRossa, D. Herlyn, D. Iliopoulos, J. Thurin and Z. Steplewski. The University of Pennsylvania Pigmented Lesion Group, Philadelphia, PA.

In an ongoing study, nine patients with Stage III melanoma were treated with a murine IgG2a monoclonal antibody (ME-361), which recognizes the melanoma-associated gangliosides, G_{D2} and G_{D3}. ME-361 fixes human complement, mediates antibody-dependent cellular cytotoxicity and inhibits melanoma growth in nude mice. Three patients were treated for five days for a total of 25mg/m^2, 3 to 50mg/m^2 and 3 to 100mg/m^2. Circulating mouse immunoglobulin was detectable in all patients for 9 to 18 days post-infusion. Human anti-mouse responses were seen in all patients temporally associated with disappearance of mouse immunoglobulin. Circulating immunoreactive ME-361 was detected only at the two higher doses. In vitro, mixing normal and patients' pre-infusion sera resulted in partial inhibition of ME-361 binding to an indicator cell, likely due to circulating gangliosides. Post-infusion immunoperoxidase analysis of frozen tumor sections were positive for ME-361 in 2/9 patients. To date, no responses and no significant toxicities have occured.

A PENILE MELANOMA CASE REPORT

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A 27 year old white male had a clinical Stage I, histologic Clark's level II penile melanoma less than .75mm thick (Breslow's method). Combining Clark's and Breslow's histologic staging schemes the melanoma was locally excised avoiding penectomy and inguinal node dissection while preserving penile function. The patient is well 54 months after surgery and there is no evidence of disease at 4½ year follow up.

56 cases of penile melanoma have been reported (mean age 56 years; range 13-78 years) with 2 patients under 30 years of age. Most workers advocate aggressive surgery and partial or radical penectomy with consideration of prophylactic lymph node dissection. Prognosis is dismal (5 year survival rate is 15%); and clinical staging has limited prognostic value.

By using both Clark's and Breslow's microinvasive histologic staging in clinical Stage I disease the extent of surgery, the need for inguinal node dissection and patient prognosis can be more clearly determined.

HUMAN MELANOMA MUTANTS SENSITIVE TO DEOXYADENOSINE ANALOGUES OR DTIC.

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Three of 8 human melanoma cell lines were highly sensitive to killing by deoxyadenosine, 2-halodeoxyadenosines, thymidine and cytosine arabinoside. Long-term treatment with deoxyadenosine led to low frequency reversion to resistance. A fourth line was hypersensitive to deoxyguanosine. Sensitivity to deoxyadenosine and its analogues was associated with inhibition of DNA synthesis, slow formation of DNA strand breaks, cell cycle block in G1/S, inhibited replication of adenovirus and absence of ecto-5'-nucleotidase and purine nucleoside phosphorylase. Adenosine deaminase and total nucleotidase activities were within the normal range. These results suggest that a proportion of melanomas could be sensitive to deoxyadenosine analogues; and could be identified by assaying adenovirus replication in primary cultures.

Melanoma cell lines sensitive to DTIC had the Mer- phenotype (methyl excision repair deficient) and lacked O-6 methylguanine transferase (MT). Mer- cells were also sensitive to methotrexate and hydroxyurea (but not deoxyadenosine) even after being reverted to DTIC resistance (Mer+) and regaining MT activity. Mutation studies indicated that Mer- cells could have a selective advantage under certain conditions.

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A total of 49 cutaneous malignant melanomas in clinical stage 1 were selected on the following additional criteria:
1) The tumor should be intact;
2) The size of the tumor should be sufficient to allow for a safe routine histological examination and at the same time provide enough material for a correct DNA determination by flow cytometry;
3) There should be no competing malignant diseases. The second criterion necessarily favored the most serious cases, and so this material cannot be regarded as representative for melanomas normally encountered.
Using the recurrence-free survival time as a measure of prognosis a univariate analysis indicates that thickness of the tumor, number of mitoses, ulceration, and age are all prognostic factors. Furthermore, it is important to note that the DNA content is of definite prognostic value.

MECHANISM OF ANTITUMORAL ACTIVITY OF CATECHOLS IN CULTURE.

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Cell lines Raji and K562, lacking tyrosinase, and two melanotic human melanoma cell lines (IRE 1 and 2) were exposed to 5×10^{-3} M ⟷ 10^{-5} M of L-Dopa (DP), Dopamine (DPA), Hydroquinone (HQ), terbutylcathecol (TBC) and phenols non substrates of tyrosinase in the presence or absence of O_2 scavenger enzymes. The stability of each substance in culture medium was assayed by HPLC.

Results showed that 1) catechols wich are substrates of tyrosinase (DP,DPA,HQ,TBC) decompose fully after 24 hrs in medium; 2) are equally toxic for non melanoma cell lines; 3) their toxicity increases when they are preincubated in medium for 24 hrs and 48 hrs before addition of cells; 4) their toxicity is significantly reduced by addition of scavenger enzymes. On the contrary, phenols non substrates of tyrosinase (resorcinol, butylated hydroxyanisole etc) are stable in medium and their toxicity is not reduced by scavenger enzymes.

It is concluded that tyrosinase does not play a major role in catechol toxicity which is probably due to some products of catechol decomposition, especially O_2 radicals acting outside the cells.

STUDIES ON THE INCIDENCE OF MELANOMA IN THE CATCHMENT AREA OF THE DERMATOLOGICAL HOSPITAL OF THE UNIVERSITY OF GIESSEN

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Like all over the world, the incidence of melanoma in the catchment area of the Dermatological Hospital in Giessen has increased during the last 15 years. The catchment area comprises an area of about 60,000 square kilometers with approximately one million inhabitants. Before 1975, the incidence rate was 2.4 per 100,000 inhabitants per year, between 1976 and 1980 it increased to 4.8, and between 1981 and 1983 it reached 10.2 per 100,000 per year, in 1984 even 11.1.

In each period, the frequency of melanoma was markedly higher in females than in males. A breakdown of the melanoma patients seen between 1981 and 1983 according to age cohorts shows that melanomas are very rare prior to the age of 15, while their incidence increases with advancing age to reach a level double that of average in persons over 75 years of age (20.6 per 100,000 inhabitants per year).

The rapid increase in the number of diagnosed melanomas after 1980 is probably due to a real increase in tumors but may also be result of an intensive education campaign. This is mainly shown by the fact that more initial melanomas were excised.

LANGERHANS CELLS ARE DECREASED ABOVE INVASIVE MELANOMA.

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Four invasive malignant melanomas, 2 metastatic cutaneous melanomas, (MCM), 3 lentigo malignas, (LM) and 3 benign nevi, (BN), were studied by immunoperoxidase staining with Leu-6, (T6) and Leu-HLA-DR, (DR). All data are mean LC/mm^2 $\pm$ s.e.m., in normal skin vs. skin over tumor. Significance was determined by paired T-test.

A decrease in T6+ LC, (317 $\pm$ 29 vs. 83 $\pm$ 37, $p < .02$, n=4), and DR+ LC, (184 $\pm$ 32 vs. 58 $\pm$ 11, $p = .03$ n=3), was observed in the epidermis above invasive melanoma. No differences in either T6+ LC, (240 vs. 254, means n=2), or DR+ LC, (244 vs. 248), were observed in 2 MCM. Similarly, no differences were observed in 3 Bn (T6+ LC; 304 $\pm$ 38 vs. 281 $\pm$ 60, $p = 0.35$. DR+ LC; 168 $\pm$ 46 vs. 188 $\pm$ 55, $p = 0.09$). T6+ LC were increased, but not significantly, in LM (301 $\pm$ 44 vs. 379 $\pm$ 29, n=3, $p = 0.08$). DR+ LC were not different in LM (261 vs. 250, means n=2).

No significant differences in dermal T6+ LC were observed in any group of lesions. However, more T6+ dermal LC were seen in LM, (1.7 LC/400X field $\pm$ .65 vs. 5.6 $\pm$ 2.5, $p = .08$).

Invasive melanomas are thus associated with fewer LC in the epidermis above them. Studies to evaluate the mechanism of this reduction are in progress.

PATHOLOGY DELAY AND MISINTERPRETATION IN MELANOMA DIAGNOSIS.

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Accurate histopathologic reporting is one of the mainstays in melanoma management. We studied the difficulties encountered in pathology interpretation in 498 melanoma patients seen during 1981-83. In 39 cases (7.8%) a pathology delay or misinterpretation had occurred. Patients in this group had an unfavorable stage at diagnosis when compared with the others ($P < 0.0001$). Reasons for the delay or misinterpretation included: (a) 5 cases with very early melanomas $\leq$ 1 mm thick were erroneously interpreted as benign nevi; (b) 10 amelanotic melanomas were initially not recognized as such; (c) partial biopsy of the primary lesion rendered proper pathology assessment impossible in 12 cases; (d) previous maltreatment without histopathologic examination giving rise to locally recurrent disease lead to a wrong initial diagnosis in 5 cases; (e) in 2 patients cryosections were initially diagnosed as benign nevi; (f) in 9 cases biopsy material from metastatic deposits, including fine needle aspiration biopsy, resulted in a wrong interpretation; (g) in 8 cases uncertainty about the diagnosis necessitated a second opinion from a colleague pathologist; (h) administrative delays of up to 2 months were encountered in 4 cases; and (i) in 2 patients no specific reason was given. Several patients showed more than one reason for the delay or misinterpretation. It is emphasized that proper biopsy technique and unremitting vigilence of the pathologist are equally essential for accurate histopathologic assessment of melanoma.

COMPARATIVE ANALYSIS OF THE METASTATIC ABILITY OF MALIGNANT MELANOMAS IN HUMAN AND ATHYMIC NUDE MICE

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Recently, we have established human cell lines (HMmK01) from nodular melanoma which has developed through a rather unique, multi-year period of stepwise tumorigenesis. The ontogeny of this tumorigenesis can be divided into four major stages : 1)nevocytogenesis (more than 6 years), 2)unmetastatic melanomagenesis (7 years), 3)low-metastatic melanomagenesis (4 years) and 4)high-metastatic melanomagenesis (2 years), leading to death at age 34.

Among isolated clones from HMmK01, an amelanotic clone (C-4-1) has high metastatic ability in nude mice. By the injection of $1x10^6$ cells from tail vein, C-4-1 has produced above 300 colonies on the lung surface in comparison to parent HMmK01 which has shown only 0-3. Furthermore only C-4-1 metastasized on the brain. Sc and ip injection also could induce much metastasis. These finding will be discussed together with our attempt to control and determine the melanoma metastasis.

It is our current thought that multiple steps of progression over a period of many years, may be related to a marked heterogeneity of the metastatic ability and to unusually high metastatic ability of heterotransplanted human melanoma cells in nude mice.

OCCULT TUMOR IN LYMPH NODES OF PATIENTS WITH CUTANEOUS MELANOMA. D-R Wen, M.D. and A.J. Cochran, M.D. University of California, Los Angeles, UCLA School of Medicine, Los Angeles, CA 90024.

Debate continues over the efficacy of elective node removal in clinical Stage I melanoma. Identification of patients with above average risk of metastases would aid selection of patients likely to benefit from this operation. In Stage II melanoma 29% of nodes, tumor-free on H&E staining contained occult metastases by immunohistology (Int. J. Cancer 34:159-163, 1984). We therefore examined 1604 nodes from 100 patients with histologically proved primary melanoma and implapable regional nodes who had elective lymphadenectomy. We reviewed the H&E stained sections and concurred that none showed metastatic melanoma. Adjacent sections were cut and stained with rabbit anti-bovine S-100 protein serum and the murine monoclonal antibody NKCI-3, using a peroxidase-anti-peroxidase technique and amino-ethyl carbazole as developer. Using this approach a total of 16 nodes from 14 of 100 patients (14%) contained single tumor cells or small groups of tumor cells. The likelihood of finding occult micrometastatic melanoma increased with increasing melanoma (Breslow) thickness (< 1.5 mm, 2/38 positive (5%), 1.5-3 mm, 6/35 positive (17%), > 3 mm, 5/27 positive (19%) and increasing depth of penetration of the dermis (Clark level) (I & II, 0/12 positive, III, 4/36 positive (11%), IV, 7/33 positive (21%), and V, 3/19 positive (16%).

APPLICATION OF MoAb HMSA-2 FOR IMMUNOHISTOPATHOLOGIC DIAGNOSIS OF AMELANOTIC AND/OR REGRESSED LESIONS OF HUMAN MELANOMA ON ROUTINE PARAFFIN SECTIONS.
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The histopathologic diagnosis of malignant melanoma (MM) with amelanotic and regressed lesions is often difficult to make. We have previously reported the establishment of a mouse monoclonal antibody, MoAb HMSA-2 which was raised against the solubilized protein of melanosomes from human MM. MoAb HMSA-2 has been shown to differentiate the neoplastic melanocytes (MC) from the normal MC on routine paraffin sections. This study evaluated the histopathologic utility of MoAb HMSA-2 for 4 cases of amelanotic and regressed primary lesions which could not be diagnosed by routine HE sections; (a) case #1 of primary amelanotic subungual melanoma with superficial spreading melanoma (SSM) type and with metastasis to lymph nodes and lung, (b) case #2 with primary amelanotic plantar melanoma with SSM type, (c) case #3 with regressed primary lesion of acral lentiginous melanoma (ALM) and with metastasis to lymph nodes and lung, and (d) case #4 with regressed primary lesion of ALM on the upper lip and with metastasis to cervical nodes. MoAb HMSA-2 provided a firm basis for histopathologic diagnosis of these 4 cases, the latter 2 cases being of particular importance because the primary regressed lesions were diagnosed retrospectively.

Chemotherapy-Cytotoxicity

INHIBITION OF HUMAN NEUTROPHIL LOCOMOTION BY A PEPTIDE GENERATED BY MELANOMA CELLS.
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It has been reported that some types of malignant cells generate inhibitors of leukotaxis which may, in part, be responsible for a host defense decrease in cancer patients. We know here, that a melanoma cell line (Beuret type, kindly provided by J.-F. Doré) do generate an inhibitor of human neutrophil locomotion as measured by the under agarose technique. The inhibitor is a small peptide which acts mainly on the speed of locomotion.

Melanoma cells were cultured for 24 hours in Mc Coy's medium to which serum, antibiotics and glutamine were not added. The cell-free culture medium was then recovered by centrifugation at 800 g for 20 min at 4°C. Inclusion of this crude extract (80µg protein/ml) in the agarose inhibited, by comparison to the controls, the locomotion of human blood neutrophils isolated by ficoll-hypaque method. Unstimulated locomotion was inhibited (60 % inhibition) as well as formyl-peptide or serum-induced locomotion (about 70 % inhibition). The crude extract, after gel filtration on sephadex G 75 and elution with ammonium carbonate (1mM) led to three Δ 320/280 nm peaks which were separately recovered. A concentration-dependent inhibitory activity was observed for the second peak whereas the two other peaks showed very slight inhibitory activity . The active peak seems to be composed of a small peptides. In conclusion, melanoma cells generate in vitro a small peptide which primarily decreases locomotion speed of human neutrophils.

IDENTIFICATION OF A DIPEPTIDASE IN MELANOMA CELLS AND SERUM
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It has been postulated that a dipeptidase in melanon cells converts an intermediate compound 5-S-L-cysteinyl-glycine-L-dopa (CGD) into 5-S-cysteinyl-L-dopa (CD) and glycine. This statement has been tested by the use of synthesized CGD as substrate and measurement of CD as product.

Method: We added 20 µl of serum, or disrupted cultured human melanoma cells to 180 µl of 0.1 M HEPES buffer, pH 6.8 at 37°C and started the reaction by mixing with CGD. After incubation for 30 minutes 0.4 ml of perchloric acid was added. Blank values was obtained by adding perchloric acid before the substrate. The amount of CD was measured by HPLC.

Result: We obtained an enzyme pH optimum at 6.8 to 7.0 with serum, and found a 3.8 µM substrate concentration of CGD to be suitable. Under these conditions the dipeptidase activity of melanoma cells was 46 pkat/10^9 cells, and for γ-GT the activity was 740 pkat/10^9 cells. In six serum samples from melanoma subjects the dipeptidase was 64±25 µkat/L which should be compared with the activity 36±8 µkat/ in healthy subjects.

Conclusion: A dipeptidase has been identified in cultured human melanoma cells and human serum. The enzyme splits the peptide bond in CGD into CD and glycine, and the CD formed can be measured by HPLC.

CHARACTERIZATION OF MELANOCYTIC CELLS ISOLATED FROM DIFFERENT STAGES OF TUMOR PROGRESSION. M. Herlyn, The Wistar Institute, Phila., PA 19104. Melanocytic cells isolated from normal skin, common acquired and congenital nevi, primary melanomas of the radial (RGP) and vertical (VGP) growth phase and metastatic melanomas retain their phenotypic characteristics when maintained in tissue culture. Genetic, biologic and immunologic markers have been developed that distinguish each cell type. Only metastatic and VGP primary melanoma cells form tumors in athymic nude mice, have nonrandom chromosomal abnormalities involving chromosomes 1, 6 and 7, and produce the highest quantities of polypeptide growth factors such as platelet derived growth factor. RGP primary melanoma cells have characteristics in vitro of both benign and malignant cells: They have a nevus-like morphology, grow permanently in culture but do not form tumors in nude mice, and they have a karyotypic abnormality involving chromosome 6. Nevus cells have a finite lifespan in culture, but they can grow in an anchorage independent manner in semisolid media and express various antigens that are present on melanoma cells. Binding of monoclonal antibodies (MAbs) generated by immunizing mice with melanoma cells was at highest levels with cells and cell supernatants of cultures from VGP and metastatic melanoma.

LETHAL EFFECT OF $^{10}B_1$-BPA-THERMAL NEUTRON CAPTURE THERAPY ON MELANOMA CELLS ENHANCED BY TYROSINE AND PHENYLALANINE DEFICIENT NUTRITION

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$^{10}B_1$-paraboronophenylalanine ($^{10}B_1$-BPA) has been shown in vivo and in vitro to be a promising boron chemical for thermal neutron capture therapy (TNCT) of malignant melanoma, although amelanotic melanoma cells were less sensitive to the lethal effects of $^{10}B_1$-BPA-thermal neutron radiation.
Aim:The present experiments were performed to find a new condition in which $^{10}B_1$-BPA accumulates efficiently in melanoma cells, leading to a more effective lethal damage to melanoma cells in $^{10}B_1$-BPA-TNCT.
Results:Incorporation of $^{10}B_1$-BPA by cultured melanoma cells was suppressed by tyrosine in dose dependent manner. Further, an enhanced lethal effects of $^{10}B_1$-BPA on melanoma cells in TNCT was demonstrated when melanoma cells were preincubated with $^{10}B_1$-BPA in the absence of tyrosine and phenylalanine.
Comments:These results suggest the tyrosinase-dependent uptake of $^{10}B_1$-BPA by melanoma cells and an enhanced therapeutic effect of the $^{10}B_1$-BPA-TNCT on melanoma-bearing subject fed with diet free of tyrosine and phenylalanine.

CLONAL ORIGIN OF B16 MELANOMA METASTASIS
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A cloned melanoma cell line HFH18-(15)-C carried continuously in culture for 8 years exhibited great heterogeneity in terms of pigmentation, DOPA and GGT (γ-glutamyltranspeptidase) reactivity and marker chromosome content. Intramuscular injections (IM) of 5×10^5 to 1×10^6 cells into syngeneic C57BL/6 mice produced tumors 1.5 to 2 cm in diameter in 3 to 4 weeks. Repeated IM injections of the cultured cells of successive tumor-to-culture passages increased the rate of tumor growth and frequency of metastases, enhanced pigmentation and enzyme activities, and improved karyotypic homogeneity.

Local IM injections of cultured cells derived from metastatic tumors did not significantly increase the incidence of metastases. Intravenous injections of cells from the parent line or cells from a lung metastatic tumor in an animal injected with this line produced numerous tumor foci in various organs. Cytogenetic analyses of 18 such lesions led to the following conclusions: (1) cells from each metastatic colony exhibited relatively homogeneous karyotypic characteristics indicating metastases are of clonal origin; (2) many parental cells with different marker chromosomes had metastatic potential; and (3) some genomes maintained homogeneity longer than others.

ACCUMULATION OF RADIOLABELED THIOURACIL IN LUNG METASTASES OF B16 MELANOMA, B.S. Larsson, K. Yamada, A. Roberto, and L. Dencker, Department of Toxicology, Biomedical Center, Uppsala University, Uppsala, Sweden.

Various thioamides, e.g. 2-thiouracil and 6-iodo-2-thiouracil, strongly accumulate in murine melanotic melanomas. They are selectively incorporated into the melanin of the tumors as false precursors. This is of potential clinical interest, since the radiation from radiolabeled thioamides might be used for the localization and possibly the therapy of malignant melanomas.

Previously we have studied the tumor uptake of thioamides in Harding-Passey melanomas, subcutaneously transplanted to mice. In the present study we have expanded the experiments to melanoma metastases to obtain more relevant information on the clinical possibilities of the technique. The accumulation and retention of C-14 and I-125 labeled thiouracil in small lung metastases of B16 melanoma was investigated in beige mice, intravenously injected with melanoma cells. The studies included whole-body autoradiography and impulse counting of excised tumor and organ pieces. The results showed that the concentration of radioactivity in the metastases was significantly higher than in normal tissues (e.g. lung, liver, kidney, eye, muscle) at all survival times (up to 5 days) after a single intraperitoneal injection. The highest tumor-/organ ratios (ranging from 6 to 124 for ^{14}C-thiouracil and from 8 to 117 for ^{125}I-thiouracil) were obtained 1-2 days after administration.

HIGH LET RADIOANALOGUES OF METHYLENE BLUE AS POTENTIAL ANTI-MELANOMA AGENTS

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It has been established that sulphur-35 radioanalogue of 3,6-(dimethylamino)-phenazationium chloride (methylene blue, MTB) which exhibits a high binding affinity for melanin, causes a significant retardation in the growth of pigmented melanomas in hamsters, but does not affect non-pigmented tumours. A radioanalogue which emits a high LET radiation might prove more efficacious. 4-[^{125}I]-iodo-MTB and 4-[^{211}At]-astato-MTB have been used in the present investigations. ^{211}At is a short-lived α-particle emitter which is produced by the ^{209}Bi(α,2n)^{211}At nuclear reaction using a 28 MeV α-particle external cyclotron beam. Autoradiographic studies with ^{125}I-MTB confirmed its co-localization with melanosomes of B16 pigmented melanoma cells. ^{211}At-MTB exhibits therapeutic properties. The therapeutic efficacy was determined by the lung colony assay in C57Bl/6 mice. Analysis of the number of colonies revealed a significant reduction in yield between ^{211}At-MTB treated and control cells.

SELECTIVE ERADICATION OF MALIGNANT MELANOMA BY A SINGLE THERMAL NEUTRON CAPTURE TREATMENT USING MELANOMA-SEEKING ^{10}B-COMPOUNDS.

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We have further advanced our Selective Thermal Neutron Capture Therapy for Melanoma, using its specific metabolic activity, to the stage where First Clinical Trials are feasible. As malignant transformation occurs in the pigment cells, they usually acquire accentuated tyrosinase activity. Thus, non-surgical treatment of melanoma can be achieved by utilizing such activity. Thermal neutrons are easily absorbed by the non-radioactive ^{10}B, resulting in the emission of α-particles and lithium atoms which release 2.33 MeV energy up to 14μ, the diameter of melanoma cells. Thus, if we selectively accumulate ^{10}B in melanoma, we can destroy it without injury to the surrounding tissues. We synthesized ^{10}B melanin substrate analogues, of which ^{10}B$_1$-p-boronophenylalanine (^{10}B$_1$-BPA) is most effective. In vitro and in vivo radiobiological analysis revealed the enhanced melanoma killing effect of ^{10}B$_1$-BPA. Chemical and prompt gamma ray spectrometry assays of ^{10}B show ^{10}B-BPA's high affinity to melanoma, such as a ^{10}B tumor/blood ratio of 11.5. After success in eradicating melanoma transplanted into hamsters, we advanced to pre-clinical studies using spontaneously occuring melanoma in Duroc pig skin. We cured, without substantial side effects, three melanoma cases, 4.6 to 12cm in diameter, by a single treatment of neutron capture, when 5g of ^{10}B$_1$-BPA and neutron 2.6 x 10^{13}n/cm^2 were given.

INFLUENCE OF CALCIUM ON THE MELANOCYTES IN THE INNER EAR.

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Inner ear melanin is distributed in well vascularized areas, especially in the vicinity of epithelial cells which are supposed to be involved in the secretion and/or absorbtion of the endolymphatic fluid. The microanalytical (LAMMA, EDXMA), histological and electronmicroscopic studies provide evidence that the melanocytes play an active role in regulating the ionic composition of the endolymph. The studies of experimental hydrops (animal model of Menière's disease) show that the melanocytes are actively involved in the Ca^{++}homeostasis of the inner ear. Moreover the melanin are able to bind and release divalent ions (Mg^{2+}, Ca^{2+}, Zn^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}), ions which are known as triggers of enzymatic activites. It seems that the increase of intracellular Ca in melanin and melanocytes, respectively, give rise to their secretory activity. Exocytosis in form of interdigitating processes and vesicles was frequently seen on cell surface facing the capillaries and occasionally in the neighbourhood of basal infoldings of the secretory cells. Furthermore migration of the melanin granules (aggregation, dispersion) and melanocytes (dislocation) was observed.

SUSCEPTIBILITY OF HUMAN MELANOCYTES TO CYTOTOXIC DAMAGE IS DUE IN PART TO INEFFECTIVE ANTIOXIDANT DEFENSES.

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We studied the effect of short term incubation with hydrogen peroxide (H_2O_2) on the viability of cultured melanocytes derived from human foreskins using an ethidium bromide/acridine orange viability assay. Compared to other cutaneous cellular targets (keratinocytes, endothelial cells, fibroblasts) also in second passage culture, melanocytes showed significantly enhanced susceptibility to lysis. The LD_{50} for melanocytes was more than 100 times lower than that of fibroblasts.

Using the specific irreversible catalase inhibitor aminotriazole (ATA), we were unable to demonstrate inhibitable catalase activity in melanocytes. In contrast, ATA increased fibroblast killing by H_2O_2, but comparable susceptibility of fibroblasts and melanocytes was only seen at toxic ATA levels.

However, the superoxide dismutase inhibitor diethyldithiocarbamate (DDC) profoundly augmented the susceptibility of melanocytes to H_2O_2 lysis. In contrast, fibroblasts showed no increase in susceptibility to H_2O_2 when treated with DDC. Thus, cultured human melanocytes are highly susceptible to oxidant damage, and although some antioxidant defenses are present, notably superoxide dismutase, the lack of antioxidants such as catalase may make these cells susceptible to oxidant attacks such as occur during inflammation.

We propose that these results may relate to the melanocyte loss seen in the phenomenon of post inflammatory hypopigmentation.

ROLE OF MELANIN IN DRUG ACCUMULATION IN THE EYE AND OCULAR TOXIC REACTION. S.Persad, J.D. Wiltshire, I.A.Menon, P.K.Basu and F.Carre. Departments of Ophthalmology and Medicine, University of Toronto, Toronto, Canada.

Several drugs form complexes with melanin. We report an in vitro system found useful for studying the effects of drugs released from the melanin-containing cells. Bovine amelanotic retinal pigment epithelial cells were cultured. Melanin isolated from human donor eyes was incorporated into these cells (MLC). The MLC were suspended in medium 199 containing chlorpromazine (CPZ). Untreated cells not loaded with melanin were used as control (UC). The supernatants were removed and the cells were washed 5 times. MLC took up more C-14-labeled CPZ than UC. Subsequent washings of the cells released more C-14-CPZ from MLC than from UC. To test drug-induced toxicity, MLC and UC were mixed with 5ug/ml CPZ and washed several times. The washings were added to suspensions of Cr-51-labeled Ehrlich ascites carcinoma cells and irradiated with a Westinghouse mercury vapor lamp. The washings from MLC produced more cell lysis (Cr-51 release) than those from UC. The phototoxicity of CPZ was found to be not due to superoxide, hydrogen peroxide or stable cytotoxic products from CPZ. These suggest that some drugs such as CPZ can accumulate in larger quantities and for longer periods in melanotic cells than in nonmelanotic cells and these drugs may subsequently be released into the extracellular fluid, thus affecting the neighbouring cells. This release mechanism may be an important factor in the ocular drug toxicity. (Supported by MRC and RP Foundation).

THE INCORPORATION OF ^{3}H-THYMIDINE (T) AND ^{3}H-URIDINE (U) IS DIFFERENTIALLY INHIBITED BY MELPHALAN IN MELANOMA AND LYMPHOBLAST CELLS AT SIMILAR LEVELS OF DNA CROSSLINKING. U. Ringborg, J. Hansson, R. Lewensohn. Department of General Oncology, Radiumhemmet, Karolinska Hospital, S-104 01 Stockholm, Sweden.

We have earlier shown that the human melanoma cell line (RPMI 8322) is relatively resistant to melphalan, compared to phytohaemagglutinin stimulated lymphocytes. The marked difference in sensitivity could be explained only partially by a moderate difference in DNA crosslinking (Anticancer Res, 5, 471, 1985). The effect of melphalan on the T and U incorporation has now been studied in these two cell populations. Cells were incubated for 30 min with different concentrations of melphalan after which the T and U incorporation was measured and related to the level of DNA interstrand and DNA-protein crosslinking, estimated by the alkaline elution technique. The following was observed: 1) both the T and U incorporation was significantly more inhibited by melphalan in the lymphocytes compared to the melanoma cells; 2) the same level of DNA crosslinking induced a significantly higher inhibition of the T and U incorporation in the lymphocytes.

Our conclusion is that the inhibitory effect of alkylating agents on the T and U incorporation may vary in different cell populations despite similar levels of DNA crosslinking. This may be one important factor of the resistance of melanoma cells to alkylating agents.

EFFECT OF MODIFICATIONS OF THE AQUEOUS SOLUBILITY OF PARA-SUBSTITUTED MONOHYDRIC PHENOLS ON TYROSINASE-MEDIATED CYTOTOXICITY IN A MODEL SYSTEM.

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Cell survival, estimated by plating efficiency, was investigated in a model system comprising human epithelial cells in layer culture exposed to a mixture of mushroom tyrosinase ($100\mu gml^{-1}$) and a series of dilutions of a set of para-substituted monohydric phenols. Several compounds were synthesised with side-chain substitutions designed to modify the lipophylic characteristics. Comparison of the water/butanol partition coefficients of these compounds with their cytotoxic potential indicates that an inverse correlation exists between aqueous solubility and cytotoxic potential.

COMBINATION OF LABELED IODOTHIOURACIL AND AN α-MSH-ANALOGUE: A POSSIBLE WAY FOR ENDO-IRRADIATION OF MELANOMAS.

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We have shown that 5-iodo-2-thiouracil (ITU) is incorporated in melanin of hamster melanoma cells. In vivo ITU was retained in tumor tissue and was exclusively incorporated in newly synthesized melanin. Our objective is inhibition of melanoma growth by means of endo-irradiation using ITU as a carrier molecule for targeting of radioactive I (125-I or 131-I). To stimulate ITU incorporation an analogue of α-MSH, viz. 4-norleucine, 7-D-phenylalanine-α-MSH (ND-MSH), was tested in vitro as well as in vivo. Cell culture experiments revealed a 70% increase of ITU incorporation in the presence of 0.1 μM ND-MSH, whereas cell proliferation was decreased. In vivo ND-MSH was tested by injecting 100 μg s.c. 24 h before i.v. administration of labeled ITU. However, no significant effect on uptake in tumor tissue could be detected as compared to control animals. A constant supply of the hormone analogue in the hamster was realized by implantation of osmotic pumps (delivery rate 1 μg/h) in the abdominal cavity 24 h before labeled ITU injection. Necropsy, performed 24 h after ITU injection, showed that ITU uptake in tumor tissue had increased by 100%, whereas uptake in other organs remained unchanged. From these results we conclude that combination of ND-MSH and ITU labeled with 125-I or with 131-I may be successful in achieving effective endo-irradiation of melanomas.

INFLUENCE OF HOST GENOTYPE ON B16 MELANOMA.

C. VOULOT, C. AUBERT. MARSEILLE, FRANCE

We have previously described an achromic transformation of B16 after transplantation to "Yellow" A^y mutant (1). Such transformation is associated with a clearcut increase of pulmonary metastase incidence. Further studies show that achromic B16, maintained on the "Yellow" mutant recovers its pigmentation but keeps its high pulmonary metastase incidence, when grafted back to the standard a/a host (2) (permanent modification observed after numerous serial transplantations). If an host environment effect may explain pigmentary transformation, a permanent (genetic) tumor modification must be considered to explain pulmonary metastase effect. This may be correlated with various findings associating A^y allele with an increased susceptibility to various tumors (3-5). So our hypothesis is a permanent (genetic) transformation of B16 by the A^y host. We have compared in C57Bl/6J strain, A^y mutation occurring at A locus (chromosome 2) with a different mutation occurring at C locus (chromosome 7) (4), cc albino-homozygotes and +C heterozygotes (6). Results obtained with C locus mutants used as hosts for B16 melanoma are very different from previous results obtained with A^y mutant host. After transplantation of B16 melanoma to cc and +C hosts typical tumor pigmentation is kept and no increase of pulmonary metastase incidence observed. We suggest a specific role for the mice mutant A^y/a chromosome 2 in the relationship between differentiation and malignancy of the B16 melanoma.

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IONIZING RADIATION (IR) SURVIVAL OF HUMAN MALIGNANT MELANOMA: INFLUENCE OF LENGTH OF INCUBATION AND NON-PROTEIN CELLULAR GLUTATHIONE (GSH) LEVELS ON APPARENT RADIOSENSITIVITY

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The time of assay is important in the assessment of cell survival based on cellular proliferative capacity as defined by a minimum colony size. We have examined the influence of the length of the incubation period on apparent radiosensitivity of human melanoma cells in an in vitro assay. Cells from melanoma cell lines, short-term cell strains and patient biopsies were plated in the upper layer of the agar bilayer, irradiated by single dose IR, and assessed periodically thereafter. Our results indicate that there is heterogeneity between human melanoma cell lines, cell strains, and patient biopsies. D_0 values ranged from 0.7 to 3.5 Gy for the cell strains. For all melanoma cells examined, D_0 values increased with the length of incubation, e.g., for patient biopsy 83-4 D_0 values were 1.3 to 2.2 Gy for cells incubated 2 to 6 weeks. Radioresistant cell strains, derived by plucking colonies from irradiated plates, have higher D_0 values than the parent cells. For example, patient biopsy 83-4 had a D_0 value of 2.1 Gy at 4 weeks, R83-4 a radioresistant clone of 83-4 had a D_0 value of 3.2 Gy at 4 weeks.

From preliminary data, D_0 values correlate with non-protein cellular GSH levels. There is heterogeneity between human melanoma cell strains. GSH levels ranged from 6.51 to 94.28 nM per million cells.

INFLUENCE OF MILD PROTEOLYSIS ON SURFACE PROPERTIES OF FORTNER'S MELANOMA CELLS

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Mild trypsin digestion is often used to dissociate solid tissues into single cell suspensions. Doubts are sometimes expressed as to whether indeed trypsinization leaves the cell surface undamaged.
The ascitic form of Fortner's hamster melanoma appears to be especially suitable in answering this question since trypsin digestion is not necessary in this case. The exudate taken from the peritoneal cavity of a hamster contains single cells ready for examination after simply washing in PBS.
Four parameters were determined: (1) the content of sialic acids in the cell membrane, (2) the partition coefficient in aqueous polymer two-phase systems, (3) the electrophoretic mobility of cells, (4) and their vitality after trypsin digestion.
It was found that (1) the amount of sialic acids decreases by 36 per cent after mild trypsinization, (2) this is reflected by the changes of the partition coefficients (3) but not by the electrophoretic mobility of cells (4) or their vitality.
It is concluded that trypsin digestion brings about changes in the chemical composition and electrical properties of the cell surface which are not detectable by measuring the electrophoretic mobility of cells or by testing their vitality.

Vitiligo-Nevi

OCULOCUTANEOUS ALBINISM IN BAMILEKE TRIBE (CAMEROON) AQUARON, R. et KAMDEM L. Centre Universitaire des Sciences de la Santé, Yaoundé, Cameroun et Faculté de Médecine, Marseille, France.

Oculocutaneous albinism is an autosomic recessive hereditary disorder frequent in Cameroon (216 subjects examined) especially in the Bamileke or Grassfield tribe : 156 persons i.e. 72 % of cases (Aquaron, Rev. Epidem. et Santé Publ., 1980, 28, 81-88). The Grassfield country occupies five of six divisions of the West Province of Cameroon with a population of about 800.000 inhabitants. The Grassfield live also in the other provinces, and the total population may be evaluated to approximately 1.500.000 (Dongmo, Thesis, Paris X, Nanterre, 1978). The Grassfield tribe is divided in mini-states or kingdoms, headed by a chief or Fon. The kingdom varies in size and in population (from 500 to 50.000 inhabitants). One hundred thirty one kingdoms have been individualized in the West province (Barbier et Nchoji Nkwi, ISH, Yaoundé, 1977). Bandjoun is the biggest kingdom with 49.000 inhabitants.

Oculocutaneous albinism has been found in 38 kingdoms. It is prevalent in Bandjoun (21 subjects) and in Balengou (22 subjects) kingdoms. The Balengou kingdom present the particularity to be governed consecutively by two albino chiefs at the beginning of the 20th century. As they were polygamist, (50 to 100 spouses and 200-300 children), spreading of the albino gene in the Grassfield tribe occured.

THE EFFECTS OF STEROIDS ON THE EXPRESSION OF AMELANOSIS IN VITILIGINOUS CHICKENS. M.L. Boyle III, S.I. Pardue, and J.R. Smyth Jr. University of Massachusetts Amherst, MA, 01003.

Modulation of the immune response via bursectomy and corticosterone therapy reduces the incidence of amelanosis in the autoimmune Smyth Delayed Amelanotic (SDA) chicken. The present study was designed to determine if testosterone proprionate (TP), diethylstilbesterol (DES), or corticosterone (CS) implants would influence immune response and amelanosis in SDA chicks. Eighty day-old chicks were implanted with TP, DES, or CS in silastic tubing to maximize the therapeutic timespan. Body weights were recorded weekly and immune response measured at 4 weeks via a sheep red blood cell hemagglutination assay. The TP group displayed significantly lower body weights ($P<.01$) and rate of gain ($P<.05$) from weeks 2-8. Amelanotic incidence in the feathers was depressed in the TP group (19%) as compared to the DES (56%), control (CON-50.0%), and CS groups (41.0%) at 8 weeks of age. DES birds displayed a significantly higher incidence of blindness due to a line associated retinal dystrophy (44%) than did the TP group (6.0%). The severity of amelanosis was also significantly higher ($P<.05$) in the DES group as compared to the TP implanted birds. TP significantly depressed antibody titers at 5, 7, and 10 days post-immunization ($P<.01$). Pooled data comparing amelanotics and normals, regardless of group revealed significantly higher titers in the affected individuals.

A MORPHOLOGIC STUDY OF MELANOCYTES IN THE HAIR FOLLICLES AND EYES OF THE VITILIGO MOUSE. Raymond E. Boissy, Gisela E. Moellmann and Aaron B. Lerner. Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Follicular and ocular melanocytes were studied in the new murine model for vitiligo, the vitiligo mouse (C57BL/6J, Ler-vit/vit). The coat of the animals lightens progressively with increasing age because of an increase in the proportion of white hairs with each hair molt (Lerner, et al., J Invest Dermatol, in press). The bulbs of the white hairs were devoid of melanocytes. Those giving rise to pigmented hairs were histologically normal but demonstrated ultrastructural degenerative changes in the pigment cells. In addition, disruption of the basement membrane underlying the melanocytes and herniation of the melanocytes into the central dermal papilla was observed at various stages of hair growth.

Newborn vitiligo mice had no uveal pigment. Pigment appeared in the iris and ciliary body by day 4 and the choroid by week 3. On day 4, conspicuous, spherical amelanotic cells appeared along the anterior surface of the iris. These cells became numerous in the ensuing weeks, and gradually acquired large melanophagosomes. They were also found in the stroma of the iris and the ciliary body, associated with necrotic melanocytes. No decrease in iris pigmentation was apparent macroscopically for the life of the vitiligo mouse. In the choroid, an amelanotic patch existed around the optic nerve. In the pigmented areas, melanocytes demonstrated degeneration and autophagocytosis.

VITILIGO-LIKE HYPOPIGMENTATION INFLUENCES FAVORABLY THE PROGNOSIS OF MELANOMA. Jean-Claude Bystryn, Darrel Rigel, Robert J. Friedman and Alfred Kopf. Kaplan Cancer Center and New York University School of Medicine, New York, N.Y.

It has been suggested that the presence of vitiligo-like cutaneous hypopigmentation favorably influences the prognosis of patients with malignant melanoma. To examine this possibility we have compared actual to predicted survival of 46 patients with melanoma and hypopigmentation, who were among 1,130 melanoma patients entered in a long-term prospective study of melanoma at the New York University Medical Center. The actual average 5 year survival of the patients with melanoma and hypopigmentation was 91%. This was significantly better than predicted (74.8%, $p=0.02$) on the basis of the risk factors present in each individual patient at the time of entry into the study. There was no significant difference in survival between patients in whom hypopigmentation was adjacent as opposed to distant from the primary melanoma.

These findings indicate that hypopigmentation is a factor that favorably influences the prognosis of melanoma and suggests that the mechanisms which inhibit or destroy normal melanocytes in patients with melanoma may also slow the growth of this cancer.

8-METHOXYPSORALEN LEVELS IN BLOOD OF VITILIGO PATIENTS AND IN SKIN, OPHTHALMIC FLUIDS AND TISSUES OF THE GUINEA PIG. Siba G. Chakrabarti, Rebat M. Halder, Beverly A. Johnson, Harold R. Minus and John A. Kenney Jr., Vitiligo Center, Department of Dermatology, Howard University Hospital, Washington, D.C., U.S.A.

PUVA therapy has become treatment of choice for vitiligo. Since only 50%-60% of the patients respond to this therapy we attempted to determine the pattern of absorption of 8-MOP in 36 randomly selected patients at timed intervals. We also attempted to determine the drug distribution in the skin and in ophthalmic fluids and tissues of the guinea pig. The drug was extracted at pH 3.0 with ethyl ether and analyzed by a reverse phase HPLC method. The lower limit of detection was 2ng and the recovery of internal standards was 89.5%. Peak blood levels in patients varied from 130ng/ml to 3892 ng/ml and was obtained at 2 or 3hrs. In the guinea pig, 2 hrs after oral administration, 8-MOP levels in ng/ml or ng/g were whole skin-379±19; epidermis-330±20; dermis-89±16; aqueous humor-441±22; vitreous gel-166±18; lens-355±15, and retina-410±26. Our results are consistent with the clinical observation that maximum response to phototherapy is obtained at 2 hrs after the drug administration at which time sufficent levels are also obtained in the skin and epidermis. The reason for non-response by some patients may be due in part to the wide variation of absorption. Hence new drugs or better drug formulation must be devised for effective treatment of non-responders. High drug levels in eye tissue and fluids in the guinea pig persist for 24hrs indicating that the eyes of the patients must be protected.

FUNCTIONAL ABNORMALITIES OF LYMPHOCYTES IN VITILIGO: P.E. Grimes, S.V.S. Golipudi, M. Ghoneum, H. Thadepalli, A.P. Kelly. Departments of Pathology and Medicine. King-Drew Medical Center, Los Angeles, California.

Previous studies from our laboratory have demonstrated a quantitative decrease in helper lymphocytes in the peripheral blood of vitiligo patients. This decrease was correlated with short duration disease. In this study, we assessed lymphocyte function by investigating their proliferative responses to polyclonal T and B cell mitogens in 11 vitiligo patients and 11 healthy controls. Peripheral blood mononuclear cells were cultured with and without phytohemagglutinin (PHA), concanavalin A (Con A) OKT3 monoclonal antibody (OKT3) and anti-immunoglobulin (Anti-Ig). Proliferation was assessed by the [3H] incorporation assay. Proliferative responses of mononuclear cells of vitiligo patients to T cell mitogens revealed a 42%, 67% and 64% decrease in mean CPM against PHA, Con A and OKT3 respectively when compared to controls ($p < .001$). The proliferative response to the B cell mitogen (Anti-Ig) demonstrated a 52% decrease ($p < .001$). These functional abnormalities did not significantly correlate with quantitative alterations in peripheral blood T cells. These results suggest functional abnormalities may precede and occur independently of quantitative T cell defects and may be relevant in the pathogenesis of vitiligo.

IMMUNOREGULATORY FUNCTION AND LYMPHOCYTE-MEDIATED MELANOCYTOTOXICITY IN VITILIGO. C. Firkins-Smith, M. Wietgrefe, M. Hordinsky, Dept. of Dermatology, University of Minnesota, Minneapolis, Minnesota. USA

Vitiligo is a common systemic disorder in which unknown factors either inhibit the production of melanin or destroy melanocytes. Proposed mechanisms for pathophysiology include neural, immune, and autodestructive. In this study, we focused on the immune hypothesis. Peripheral blood lymphocytes (PBL) were obtained from 20 white patients (12F, 8M) and controls for the following studies: immunoglobulins G, A, M, E, lymphocyte proliferation to the mitogens phytohemagglutinin, Concanavalin A, pokeweed, and the lymphocyte subsets identified by the monoclonal antibodies OKT3, OKT11, OKT4, OKT8, and B1. In addition, we established a lymphocyte-mediated melanocytotoxicity assay using human PBL as effector cells, cultured human melanocytes as target cells and human PBL from controls and patients and cultured melanocytes as stimulator cells. Five patients with vitiligo were studied. Melanocytotoxicity was measured by determining ^{51}Cr release from target cells. Immune function studies demonstrated no significant differences between patients and controls. In the cytotoxicity assays performed, significant differences between melanocytotoxicity by PBL from controls and patients were demonstrated in only one patient, a female with active vitiligo and documented IgA deficiency. We conclude that there is no significant immunoregulatory abnormality present in white patients with vitiligo. However, the results of the cytotoxicity assays suggest direct killing of melanocytes by lymphocytes may occur in some patients with vitiligo.

DYSPLASTIC NEVI ARE PRECURSORS AND RISK MARKERS OF SPORADIC MELANOMA. D. Guerry, D.E. Elder, W.H. Clark, E. Bondi, and M. VanHorn. The University of Pennsylvania Pigmented Lesion Study Group, Philadelphia, PA.

To determine the prevalence of dysplastic nevi (DN) in patients with sporadic melanoma (no melanoma in the extended nuclear pedigree) we counted and mapped all normal and abnormal nevi in 105 patients. Demographic data, type and microstage of the primary melanoma, the presence of a histologically observable precursor, and oculo-cutaneous phenotype were also recorded. 45 (43%) of patients had ≥1 DN away from the primary site. 98% of these patients had ≥1 DN on the trunk/buttocks. 7% of patients had dysplasia only at the primary site and 20 (19%) had dysplasia at the primary site and DN. 40/45 (89%) of patients considered to have DN and 11/11 patients with clinically normal nevi had their lesions histologically confirmed. Patients with DN had a mean of 14 abnormal nevi and more normal nevi than those without dysplasia (37-v-16, $p < .0001$). Patients with DN differed in eye color (80%-vs-42% had blue/grey irides, $p = .0002$) and age (43-v-51 years old, $p = .007$), but did not differ from those without DN in sex, microstage, or location of the primary. The high prevalence of melanocytic dysplasia in these patients (50%) in comparison to "normal" population members (range 1.8-8%) suggests that melanocytic dysplasia is both a precursor and a risk marker for sporadic melanoma.

OCULAR FINDINGS IN VITILIGO. Rebat M. Halder, Claude L. Cowan, Jr., Pearl E. Grimes, Siba G. Chakrabarti, John A. Kenney, Jr., Div. of Ophthalmology, Vitiligo Center and Department of Dermatology, Howard University College of Medicine, Washington, D.C., U.S.A.

Vitiligo, an acquired loss of melanocytes in the skin has been found to affect melanocytes in other organs including the eye. We examined 156 patients with vitiligo for ocular abnormalities. An ocular examination was performed including visual acuity, biomicroscopy, applanation tonomety and dilated fundoscopy. A two to three ratio of white to black patients allowed us to evaluate the role of race in the occurrence of ocular disturbances. A large percentage (40%) of all patients showed some degree of fundal pigment disturbance including pigment clumps, focal hypopigmented spots, and choroidal nevi. While it is possible that some of these fundal abnormalities represented sequelae of previous inflammation, active uveitis was documented in only two patients. Racial variations were found with an increased incidence in whites of choroidal nevi ($p = 0.001$) and depigmentation of the iris pigment border ($p = 0.0012$). Thus, this study (1) supports some of the earlier reported findings of ocular abnormalities in vitiligo; (2) reports a lower incidence than other studies of active uveitis in patients; (3) adds new information in racial variations in ocular findings in vitiligo.

CIRCULATING T LYMPHOCYTES AND INTERLEUKIN-2 ACTIVITY ARE DECREASED IN VITILIGO. Rebat M. Halder, Curla S. Walters, Beverly A. Johnson, Siba G. Chakrabarti, John A. Kenney, Jr., Vitiligo Center and Departments of Dermatology and Medicine, Howard University College of Medicine, Washington, D.C., U.S.A.

Vitiligo is an acquired, sometimes inherited loss of melanocytes in skin and other organs, the etiology of which is not clear. We attempted to study some aspects of cellular immunity in vitiligo. Twenty-five patients with vitiligo and 25 healthy controls were evaluated with flow cytometry to compare percentages of circulating T lymphocytes. Using OKT3 and OKT 4 monoclonal antibodies, it was determined that mean total T lymphocytes and helper T lymphocytes were depressed in patients compared to controls ($p<0.001$). These findings may be due to decreased activity of a T lymphocyte-stimulating factor, such as interleukin-2 (IL-2). We then investigated IL-2 activity in thirteen of these patients and 18 controls. IL-2 activity was determined by screening the activity of supernatant harvested from incubated lymphocytes of subjects on generated IL-2 dependent T lymphocytes as measured by thymidine uptake at eight dilutions from 1:2 to 1:256. IL-2 activity was depressed in patients compared to controls at all dilutions ($p<0.025$). Thus, we have found that cell-mediated immunity is subject to some defect in regulation in vitiligo. Whether this can be associated with the pathogenesis of the disease is not clear, however the data supports an autoimmune aberation occurring in vitiligo.

HUMAN PIEBALDISM WITH SPONTANEOUS AND PUVA-INDUCED REPIGMENTATION: AN ELECTRON MICROSCOPIC STUDY. Toshio Hamada, Kazuyoshi Fukai, Masamitsu Ishii, Jun-ichi Kitajima and Yu'ichi Terao.
Department of Dermatology, Osaka City University Medical School, Osaka, Japan.

Piebaldism is a congenital circumscribed hypomelanosis of the skin and hair in a pattern distinctive both for its distribution and for the islands of pigmentation, which are present from birth and do not change in size throughout life. We report a case of piebaldism, nine-year-old Japanese girl, has been followed since three-month-old, in which hyperpigmented macules have been appeared spontaneously on the hypomelanosis without the islands of pigmentation at birth, and more some induced by PUVA therapy.

In the hypomelanotic skin, electron microscopy revealed that considerable number of melanocytes were recognized. Most of melanosomes in the melanocytes were stage II to III and formed melanosome complex in the dendrites. However, there were no melanosomes in the surrounding keratinocytes. The melanocyte-keratinocyte transfer of melanosomes seemed to be impaired. In the another area of hypomelanotic skin, there were no melanocytes and increased indeterminate cells. In the islands of pigmentation within hypomelanotic skin, spherical or irregular in shape and partially melanized melanosomes, and also ellipsoidal lamellar normally melanized melanosomes were observed.

ULTRASTRUCTURAL STUDIES OF HALO CONGENITAL NEVUS. Toshio Hamada, Jun-ichi Kitajima, Masamitsu Ishii, Kazuyoshi Fukai and Miyako Chanoki.
Department of Dermatology, Osaka City University Medical School, Osaka, Japan.

Ultrastructural studies of halo congenital nevus were performed. A eight-year-old Japanese boy has been developed leukoderma concentrically since one year ago, on the surroundings of the round brownish black pigmented plaque of right chest since at birth. Histopathological features of the pigmented plaque were compound nevus. No significant inflammatory response was present in the dermis. Electron microscopic findings showed melanocytes in halo epidermis decreased in number. In the remaining melanocytes, normal melanosomes decreased and a number of spherical and granular abnormal melanosomes were observed. Langerhans cells in the basal layer of halo epidermis increased and were frequently in contact with lymphocytes. Some nevus cells in the basal layer of epidermis showed pyknotic nuclei and vacuolated cytoplasm. In the dermis, no degenerated nevus cells were observed. Langerhans cells on the epidermis overlying the nevus considerably increased in number and were seen in contact with several nevus cells. They contained numerous Langerhans granules, lysosomes phagocytosed melanosomes and many vacuoles due to the enlargement of endoplasmic reticulum. Although inflammatory reaction was scarcely observed in halo congenital nevus, these ultrastructural findings suggested a relationship to some type of immunological mechanism for the degeneration of epidermal nevus cells.

MELANOSOMAL ANTIGEN EXPRESSED ON THE CELL SURFACE OF PIGMENT CELLS AS A POSSIBLE TARGET IN AUTOIMMUNE VITILIGO

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Vitiligo is known to be frequently associated with disorders of the immune system and supported as an autoimmune disease by the detection of autoantibodies to melanocytes. Premelanosome and melanosome had been found to possess the antigenicity revealed by the blastogenic responses of lymphocytes from melanoma-bearing hamsters. The immunological role of such melanosomal antigens in vivo has remained to be investigated. Thus, we have established anti-melanosome-associated monoclonal antibody (MoAb) that reacts with premelanosomes, melanosomes and GERLs in pigment cells. This MoAb has been found to react also with the cell surface of pigment cells regardless of their oncogenic differentiation status. To reveal a possible role of cell surface expressed-melanosomal antigen, we have examined ADCC of this MoAb, using spleen mononuclear cells as effectors, against cultured-melanoma cells and -normal melanocytes. We have observed significant ADCC of this MoAb against both human melanoma cells and normal melanocytes, comparing with controls. Biological and biochemical characterization of ADCC-induced cytotoxic changes in normal and malignant pigment cells will also be discussed. The above would indicate the possibility of antigen presentation in vivo by melanosomes from pigment cells in the induction of vitiligo depigmentation.

PIGMENTARY DEMARCATION LINES: A POPULATION SURVEY, William D. James, MD, E. Ann Mountcastle, MD, Jan Carter, MD, O. G. Rodman, MD, Walter Reed Army Medical Center, Washington, D. C.

The object of this study was to document the incidence of all five types of pigmentary demarcation lines (PDL) in both Black and White populations, as well as to determine their age of onset and the effect of pregnancy on their appearance. A prospective random study of 200 adults, 100 newborns, 75 children and 100 pueperal women was accomplished. Equal numbers of Black and White patients were screened, and the study had an equal sex distribution as well.

Seventy-nine percent of Black female adults have at least one type of PDL, with types A and B being present in over 50% of the cases. Pregnancy led to the new appearance or accentuation of type B lines in 7 of 50 Black women (14%) and may account for the high incidence of this line in adult Black females. Seventy-five percent of Black males had at least one PDL, with type C being most prevalent. Fifteen percent of White males had one. PDL can be observed in the newborn period. Their incidence increases early in life. Several unique pigmentary patterns wer observed.

Knowledge of normal pigmentation patterns aid in distinguishing normal skin markings from those associated with disease states. An example is the type E line, which can be confused with ash-leaf macules of tuberous sclerosis. This study defines PDL in a large randomly selected population.

ESTABLISHMENT OF A COMPUTED IMAGE ANALYZING SYSTEM FOR QUANTITATIVE CHARACTERIZATION OF MELANOCYTES IN CAFÉ-AU LAIT MACULES OF NEUROFIBROMATOSIS PATIENTS:
Osamu Ishida and Kowichi Jimbow
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The nature of hypermelanosis in café-au lait (CAL) macules in patients of neurofibromatosis (NF) is still unsettled. Previously the CAL macules have been characterized by the population density of melanocytes (MC). However, some studies found a significant increase in MC populations compared to normally pigmented skin while others observed such a difference in only a few cases. This study characterizes the numerical and morphological changes of MC in CAL macules of NF patients by establishing a computed image analyzing system for split dopa preparations and by comparing the computed images of MC in CAL macules of light brown, brown and dark brown pigmentation. The parameters included (a) area and perimeter of the whole MC, (b) area, maximum length, vertical length and perimeter of MC cytoplasm and (c) maximum length, breadth and number of MC dendrites. We found (a) that the MC populations in the light brown and brown macules are basically similar to those of normally pigmented skin and are characterized by an increase in the area and perimeter of the cytoplasm and dendrites and (b) that the MC in the dark brown macules increased their number (2 folds) and in contrast, revealed a decrease in the area and perimeter of cytoplasm and dendrites, though the number of dendrites did not change.

PIGMENT SYSTEM CHANGES IN PRADER-WILLI SYNDROME. RA King, CM Bendel, GL Wiesner, D Arthur, D Creel, University of Minnesota, Minneapolis and VA Hospital, Salt Lake City, Utah.

The Prader-Willi Syndrome (PWS) is a common congenital disorder characterized by obesity, mental retardation, short stature and hypogonadism. The etiology is unknown but approximately 50% of PWS have an interstitial deletion of the distal long arm of chromosome 15.Hypopigmentation has been described but has not become a recognized feature of the syndrome. We have studied 29 nonalbino individuals with PWS. Clinical hypopigmentation was present in 48% using criteria that included skin type, iris translucency, and family history. Those with hypopigmentation had a lower mean hairbulb tyrosinase activity (0.49 ±0.51 pmol/120min/bulb, range 0-1.53) than those normally pigmented (0.69 ±0.50, range 1-1.36). Cysteinyldopa excretion was low in both groups, with 12/13 hypopigmented and 6/15 normally pigmented PWS excreting no detectable cysteinyldopa. There was a significant correlation between the presence of hypopigmentation and the deletion of chromosome 15. Three of 4 hypopigmented PWS had abnormal visual evoked potentials which indicate a misrouting of the optic system identical to that found in oculocutaneous albinism. The melanocytes from 1 hypopigmented PWS had abnormally large melanosomes with a disrupted internal matrix. Hypopigmentation appears to be a common feature of the PWS phenotype, and there is a gene (or genes) on chromosome 15 which appears to regulate pigment formation.

MoAb HMSA-2 AS A DIFFERENTIATION MARKER FOR DYSPLASTIC MELANOCYTIC NEVI FROM COMMON MELANOCYTIC NEVI ON ROUTINE HISTOLOGY SECTION:
Kazuo Maeda, Kaori Yamana, Kowichi Jimbow, Yutaka Akutsu, Hiroyuki Takahashi, Department of Dermatology Sapporo Medical College, Sapporo, Japan

The nature and histogenesis of dysplastic melanocytic nevi (DMN) are still unsettled. Previously we have shown (a) that MoAb HMSA-2 (human melanosome associated antigen) identifies neoplastic melanocytes, (b) that its reactivity is different in common melanocytic nevi (CMN) and malignant melanoma (MM), and (c) that pigment cells in the epidermis of CMN do not react with MoAb HMSA-2 while those of dermal nevus cells do react with it strongly (J Invest Dermatol 84:319-320, 1985). This study reports the reactivity of MoAb HMSA-2 with the cells of typical DMN (both clinically and histologically) on formalin fixed and paraffin processed specimens. We found (a) that the pigment cells in the epidermis behave differently with MoAb HMSA-2 in CMN and DMN, (b) that the dysplastic melanocytes (MC) reveal many similarities with those MC of superficial spreading melanoma and (c) that the epidermal pigment cells of DMN are positive while those cells of CMN are negative with MoAb HMSA-2. In addition, the melanosomes transferred into keratinocytes were strongly positive with MoAb HMSA-2, indicating that the melanosomes in DMN are abnormal in both melanocytes and keratinocytes. It is likely that MoAb HMSA-2 is a new class of MoAb which differentiates DMN from CMN on routine paraffin-sections.

THE DEVELOPMENT OF HYPERPIGMENTED TRANSVERSE STREAKS IN THE SCARS OF EXCISED MELANOMAS. Brian T. Morris, Arthur J. Sober, Department of Dermatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA U.S.A..

The initial treatment of clinical Stage I cutaneous melanoma is surgical excision. In many of our patients, wound healing resulted in the production of tan-brown pigmented streaks that stretched across the nascent scar perpendicular to the long axis.

To determine the frequency of this finding and associated features, 50 sequential melanoma patients were examined along with their records to obtain age, sex, melanoma site, type, level, thickness, duration of follow-up, presence of freckles, dysplastic nevi, and pigmented streaks in the melanoma scar. Data was analyzed employing chi-square analyses.

68% (34/50) of the patients demonstrated pigmented streaks and 92% (46/50) were frecklers. There was a statistically significant association between the presence of freckles and the pigmented streaks. Whether this association is causative remains to be determined. There was also a statistically significant relationship between length of follow-up and the appearance of the pigmented streaks ($\leq$9 mo.-25%,>9 mo.-82%). None of the other factors correlated with the presence of the streaks and no cases of recurrent melanoma have arisen in the scars. Streak pigmentation was enhanced by long wave UV light, suggesting an epidermal location for the hyperpigmentation. Similar streaks were also observed in non-melanoma scars in some of these patients.

RED ALBINISM M. Mizoguchi, S. Sakata, Y. Kawaguchi, H. Sato, Y. Kawa, S. Ito# and Y. Hori*. Dept of Dermatology, Teikyo Univ., Tokyo, #Fujita-gakuen Univ., Aichi, and *Yamanashi Medical College, Yamanashi, Japan.

Oculocutaneous albinism was divided into heterogeneous groups, one of which was categorized red albinism. The red albinos are known to have pheomelanin, however, none of them have been examined ultrastructually or chemically. A 28-year-old Japanese man was suspected of having atypical tyrosinase positive albinism because of his reddish brown hair and rather white skin for a Japanese. During his childhood, he had whitish yellow hair and white skin which had gradually gained brown color with increasing age. He hasn't had visual impairment since his birth, but had slight photophobia when he was a little child. His parents were normally pigmented and were not known to be consanguineous. The pedigree data suggested that none of his relatives had red hair. Ultrastructurally, his hair and skin had pheomelanosomes. Microanalysis of eumelanin and pheomelanin in hair by chemical degradation and liquid chromatography indicated that PTCA (eumelanin indicator) was 16ng/mg, and that AHP (pheomelanin indicator) was 350ng/mg. Based on these findings he was diagnosed as having red albinism.

10 YEARS OBSERVATIONS ON THE EFFECT OF AZELAIC ACID ON LENTIGO MALIGNA.
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Ist.Derm.St Gallicano,Rome; St Mary's Hosp.Medical School, London; Dermatol.Clinic,Univ.Turin,Italy.

Since 1976 50 patients with lentigo maligna have been treated with topical (a 20% cream) azelaic acid.

All cases showed positive results up to complete regression. Time to complete resolution varied between 4 and 12 months, depending upon the type and extent of the lesion and the consistency of regular application of the cream. During treatment, histology and electron microscopy confirmed reduction in number, massive lipid degeneration and destruction of atypical melanocytes, with return towards normal organisation of the epidermis, reconstruction of basal lamina and diseappearance of lymphocytic response. No side effect of either toxic or allergic nature, and no local or focal hypopigmentation was observed. These results have recently been fully confirmed by Leibl et al in 7 patients with lentigo maligna.

In our series, 27 patients are now 5 to 10 years post cessation of treatment. The affected areas continue to appear normal and at this time biopsy revealed normal cutaneous architecture and normal melanocytes. In 11 cases slight focal relapses developed; reapplication of the cream resulted in complete healing.

STUDIES ON DEPIGMENTATION AND IMMUNITY IN C57BL/6 VIT•VIT MOUSE. J. Nordlund, S. Amornsiripanitch, L. Rheins, Department of Dermatology, University of Cincinnati College of Medicine, Cincinnati, OH.

One strain of C57BL/6 mice has a recessive gene (vit) causing loss of pigment cells (PC) in the skin, hair and eyes. The process begins at 4 weeks of age and is near completion at 6 months. By EM, the skin is depleted of PC and the keratinocytes show evidence of degeneration. No inflammatory cells are detectable. These findings resemble human vitiligo. Vitiligo patients have depressed reactivity to allergens like dinitrofluorobenzene (DNFB). We compared the response to DNFB of vit/vit mice to congenic C57BL/6 age-matched controls. Like humans, vit/vit mice have a markedly diminished reaction to DNFB. The Ia+ cells (LC) were 322 ± 25 (cells/mm^2) in vit/vit and 396 ± 25 in normal. The numbers of Thy1.2+ cells were 482 ± 18 in vit/vit and 541 ± 14 in normal. Allogenic skin grafts were rejected on day 9 post-transplant by vit/vit mice, not different from the controls. Dermal delayed hypersensitivity (DTH) to sheep red blood cells in 6 week vit/vit was 71.5 ± 4.9% and 77.2 ± 1.9% in normal. The thymus and spleen are about 50% larger at 6 weeks in vit/vit vs. normal controls. We conclude that vit/vit mice have lost epidermal immune reactivity but have a normal central immune system. These data suggest the loss of PC may not be caused by the immune system but may be responsible for the depressed cutaneous immune response. Vit/vit mice are a new important model to study the interaction of melanocytes and immune cells.

APPROACHES INVOLVED IN STUDYING HYPERPIGMENTATION & DEPIGMENTATION REACTIONS OF HUMAN SKIN. MA Pathak, Dermatology Dept, Harvard Med School, Boston, MA.

Processes associated with the increase or decrease of skin pigmentation are best understood by studying the morphologic changes in perikarya and dendrites of melanocytes and the biochemical reactions involving formation, melanization (tyrosinase activity), transfer, and degradation of melanosomes. Light and electron microscopic observations derived from studying the process of increased pigmentation (e.g., tanning reaction stimulated by UVB or PUVA) and the process of decreased pigmentation (depigmentation by 4-isopropylcatechol, 4-hydroxyanisole, etc.) reveal the importance of studying three approaches: a) a numerical count of melanocytes per unit area and their functional state (proliferative, quiescent, and/or damaged) related to S-phase activity of cell cycle; b) the structural changes in melanocytes and melanosomes and the determination of increased or decreased tyrosinase activity; c) determine whether free radicals and reactive oxygen species ($^1O_2$, $^{\bullet}OH$, and O_2^-) are generated which induce metabolic alterations in DNA, mitochondria, and/or cell membranes. Stimulation or inhibition of pigment cell activity involves direct and indirect actions. Direct action includes events ascribable to DNA (e.g., formation of thymine dimers, psoralen-DNA adducts, or inhibition of DNA synthesis and mitosis). Indirect action involves metabolic perturbations (light and chemical) involving increased or decreased synthesis of tyrosinase and generation of reactive oxygen species that evoke lipid oxidation and cell membrane damage.

A PROFILE OF CYTOTOXIC DAMAGE TO HUMAN MELANOCYTES PRODUCED BY VITILIGO PATIENT'S SERA IN VITRO. D.A. Norris, R.M. Kissinger, J.C. Bystryn, G.M. Naughton, Depts. of Dermatology, Univ. of Colorado, Denver, CO. and New York University, N.Y., N.Y.

There is considerable debate whether the anti-melanocyte antibodies found in vitiligo patient's sera are related in any way to the disappearance of melanocytes which characterizes this disease. We have shown that sera containing such antibodies from vitiligo patients can induce the immunologic lysis of melanocytes mediated by complement and by antibody dependent cellular cytotoxicity (ADCC).

Human foreskin melanocytes grown in slide chambers were incubated with combinations of heat-inactivated test sera, human complement and ADCC effectors. Cytotoxicity was measured by a single blinded observer using an ethidium bromide/acridine orange assay. Ten vitiligo sera and ten normal sera were compared to a known positive anti-melanocyte rabbit sera.

Vitiligo patient's sera, but not normal sera, produced significant ($p<.001$) complement-mediated lysis and ADCC in both 4 and 16 hour experiments comparable in magnitude to that produced by the positive rabbit serum. Cell detachment as well as cell death was noted with some sera plus complement. Importantly, cultured human skin fibroblasts were not lysed by vitiligo sera and complement.

The lysis of melanocytes by two different antibody-dependent mechanisms may be important in the melanocyte destruction seen in vitiligo.

ELECTRON MICROSCOPY OF DYSPLASTIC NEVI BASED ON THE CONCEPT THAT MELANOCYTIC NEVI ARE INTRAEPIDERMAL STRUCTURES. A. Pawlowski and P.J. Lea, Departments of Medicine and Anatomy, Faculty of Medicine, University of Toronto, Ontario, Canada M5S 1A8.

The membrane surrounding nevi, is a basement membrane derived from the epidermal- dermal junction. The nevus cells were found to protrude into the dermis from the interfollicular epidermis, epidermal pegs and hair follicles.

In serial sections of dysplastic nevi, the stratum malpighii was two to three cells thick, with large, multiple blood vessels just below the basement membrane. The basement membrane around the nevus nests and cells varied considerably in thickness and structure. Melanosomes in dysplastic and other nevi did not show any obvious differences. Multiple lipid droplets were found in the melanocytes of dysplastic nevi.

Ultrastructural differences between dysplastic and other melanocytic nevi, may be useful in understanding the possible change from the benign lesion to the malignant tumor.

MELANOSOMAL ALTERATIONS IN DYSPLASTIC MELANOCYTIC NEVI: A QUANTITATIVE ULTRASTRUCTURAL INVESTIGATION. AR Rhodes, Y Seki, RS Stern, TB Fitzpatrick. Department of Dermatology, Harvard Medical School, Boston

Abnormal melanosomes are common in cutaneous melanoma. Identical melanosomal abnormalities occur in intraepidermal melanocytes of dysplastic melanocytic nevi (DMN). To further investigate the significance of melanosomal alterations, we used transmission electron microscopy at 24,400X to assess melanosomes as normal or abnormal in a sampling of intraepidermal melanocytes >5 mμ in diameter in 4 categories: 2710 melanosomes in 28 melanocytes from 5 DMN from 5 individuals (2 with melanoma, 1 with a family history of melanoma, and 2 with neither), 3270 melanosomes in 33 melanocytes from 5 superficial spreading melanomas (SSM) from 5 additional individuals, 1572 melanosomes in 30 melanocytes from 5 typical acquired nevomelanocytic nevi (NMN) from yet another 5 individuals, and 715 melanosomes in 27 melanocytes from 5 specimens of normal skin (NS) adjacent to DMN. The mean percent (±SD) of abnormal melanosomes per melanocyte (ratio of abnormal to total melanosomes assessed, x 100%) in DMN (44±23%) was 7 times greater than that in NMN (6±7%) and 22 times greater than that in NS (2±5%) ($p<0.001$, both comparisons), but only 80% of that in SSM (57±19%)($p<0.02$). The increased concentration of abnormal melanosomes in intraepidermal melanocytes of DMN and SSM could not be attributed to area of the perikaryon or nucleus, or ratio of nuclear area to cytoplasmic area. Melanosomal alterations appear to be a useful marker of atypicality in melanocytic tumors.

DEVELOPMENTAL DEFECTS ASSOCIATED WITH HYPOMELANIC MUTATINS IN THE DOMESTIC FOWL. J. R. Smyth,Jr., M.L. Boyle III, S. M. Damien and S.L. Pardue. Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA 01003 USA.

Pleiotropies involving embryonic and early postnatal development were determined and compared for four hypomelanizing mutants in the fowl. The mutations included were the autosomal C-alleles, c^a, a tyrosinase-negative (ty-neg) albino, and c, a partially tyrosinase positive (ty-pos) with pigmented eyes and white plumage; s^{al}, a sex-linked ty-pos partial albino; and pk, an autosomal ty-pos with pink eyes and slightly diluted plumage color. Comparisons at each loci were between hypomelanic phenotypes and normal heterozygous sibs. Both c^a and s^{al} albino embryos showed reduced growth rate at 7 ($P<.05$) and 14 ($P<.01$) days and increased late embryonic mortality ($P<.01$). At hatching they also had shorter down length ($P<.01$), while along with pk they showed higher incidences of nares and hock inflammation ($P<.01$) and yolk sac protrusions ($P<.01$). Postnatal growth rate was significantly reduced for c^a, s^{al} and pk chicks through days 3,2 and 24 respectively. The recessive white (c) phenotype differed significantly from c^a but not C^+ sibs, for all comparisons. Therefore, close similarities exist between certain developmental pleiotropies associated with hypomelanic mutants known to involve different genetic pathways, i.e., ty-neg versus ty-pos. This suggests that either the presence of functional pigment cells, or melanin itself, may contribute to normal development of specific tissues in the chick embryo.

THIOREDOXIN REDUCTASE FOR FREE RADICAL REDUCTION ON THE SKIN IN DIFFERENT HYPOPIGMENTATION DISORDERS. K.U. Schallreuter, M.D., Maria K. Hordinsky, M.D. and John M. Wood*, Ph.D. Departments of Dermatology and Biochemistry*, University of Minnesota.

We studied thioredoxin reductase activity as an indicator of free radical defense on the skin of 30 healthy human volunteers (different skin type I-VI Fitzpatrick Classification). Furthermore, we examined 11 cases of untreated vitiligo, 12 cases of the inherited pigmentation disorders piebaldism and albinism, and 5 cases of post-lesional leukoderma.

A membrane associated thioredoxin reductase has been discovered in human epidermis which is active in the reduction of free radicals. A rapid and accurate in vivo bioassay for this enzyme has been developed by using a spin-labelled quaternary ammonium salt (quat) as a free radical substrate. The results from the 30 normal healthy volunteers showed that enzyme activity could be correlated with pigmentation and skin type. However, environmental factors and poor metabolic status significantly decrease thioredoxin reductase activity. In vitiligo we found approximately a three fold decrease in specific activity for thioredoxin reductase compared to the normal skin of the same donor. Patients with piebaldism, and post-lesional leukoderma presented no differences in specific activity.

ABERRANT MELANOGENESIS AS A TOOL FOR ELECTRON MICROSCOPIC DIAGNOSIS OF DYSPLASTIC NEVI:
Hiroyuki Takahashi, Takashi Horikoshi, Yutaka Akutsu, Kazuo Maeda, Kaori Yamana, K. Jimbow
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In our previous report (Cancer 56:111-123, 1985), we have shown that fine structural features of melanosomes (MS) in melanocytes (MC) of dysplastic nevi (DN) are aberrant and quite different from common melanocytic nevi. This study is an extension of the previous one, in which attempts are made to characterize the fine structure of MSs transferred to keratinocytes (KC), with 11 additional cases of "typical" DN, and to further define abnormal melanogenesis, with 2 other "atypical" cases. These 2 cases were atypical because of lack of lamellar fibroplasia and mesenchymal reaction, one of the major criteria proposed by Elder et al. We found (a) that the fine structures of MSs in MC of additional 11 cases are abnormal and identical to those reported previously, (b) that the MS structures in KCs in all DN cases are also aberrant and largely in the forms of spherical-granular or incompletely lamellar patterns, (c) that MSs transferred into KCs under these conditions appear to occur even before the stage IV of MS development, and (d) that MS structures in "atypical" cases are also aberrant in both MCs and KCs. Thus it is likely that the melanogenesis in DN is aberrant and that the fine structural features of MSs are so unique in DN that they can be a good marker for diagnosis of DN.

URINARY EXCRETION OF SOME MELANIN-RELATED COMPOUNDS IN INDIVIDUALS WITH DIFFERENCES IN MELANIN PIGMENTATION

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Urinary excretion of 5-hydroxy-6-methoxyindole, 5-hydroxy-6-methoxy-indole-2-carboxylic acid, 3,4-dihydroxyphenylalanine and 5-S-cysteinyldopa was measured in urine samples of four groups of persons: (1)patients with vitiligo, (2) patients with albinism, (3) people of Hindustani origine (4) the group of Caucasoids. Indole substances were determined with gas chromatography - mass spectrometry, the concentration of 3,4-dihydroxy-phenylalanine and 5-S-cysteinyldopa was measured with the use of high-performance liquid chromatography. The results were expressed in umol/mmol creatinine. They indicate that, of all measured melanin-related substances, 5-hydroxy-6-methoxyindole-2-carboxylic acid is the best marker of melanin formation in skin melanocytes.

UNUSUAL LENTIGINES IN A PATIENT WITH VITILIGO AFTER LONG-TERM PHOTOCHEMOTHERAPY (PUVA). K. Yonemoto, K. Kamimura, and S. Kondou, Kitasato Univ. School of Medicine, Sagamihara, Japan.

Wide spread freckles, one of the possible long-term side effects of photochemotherapy (PUVA), have been reported in patients with psoriasis. We encountered a patient with vitiligo who developed clinically similar freckles in the site of vitiligo following long-term PUVA therapy.

The patient, 13 year-old Japanese boy, had been treated with PUVA for 4 years. He had received more than 1500 J/cm^2 of PUVA. Not only repigmentation occured but unusual lentigines were observed. These lentigines were irregular in outline and were darkly and unevenly pigmented.

Histologically, these freckles consisted of a lentiginous proliferation of active melanocytes, which were relatively large and sometimes atypical. Ultrastructurally, melanocytes in the lentigines revealed active melanogenesis and had numerous dendrites. Huge melanosome complex was observed in basal keratinocytes in the lentigines. These findings are substantially similar to those observed in PUVA lentigines which appeared in the white patients with psoriasis. No reports of PUVA lentigiens have been obtained in Japanese. The mechanisms of the PUVA lentigines observed in vitiligo require further evaluations.

ELEVATED URINARY DOLICHOL SECRETION IN HERMANSKY-PUDLAK SYDNROME - AN INDICATOR OF LYSOSOMAL DYSFUNCTION. CJ Witkop Jr, LS Wolfe†, SX Cal *, JG White, D Townsend, KM Keenan. U of Minnesota, Minneapolis, MN, USA; * Mt. Sinai School of Med, NY, NY, USA: † Montreal Neurological Inst, McGill U, Montreal, Quebec, Canada.

Hermansky-Pudlak syndrome, a triad of albinism, platelets lacking dense bodies and storage of ceroid-like material in tissues occurs approximately once in 2000 Puerto Ricans. The manifestations of storage disease are variable, including ulcerative colitis, restrictive lung disease, kidney failure and cardiomyopathy. The autofluorescent material stored in Hermansky-Pudlak syndrome is histochemically similar to that stored in neuronal ceroid-lipofucsinosis. The material in neuronal ceroid-lipofuscinosis contains dolichols which are components of lysosomes, and patients excrete high levels of urinary dolichols. This study of 49 Hermanksy-Pudlak patients found that urinary dolichols are elevated in those patients with evidence of ceroid storage in their kidneys but are not elevated when storage occurs in tissues other than kidney. The excretion of ceroid was not influenced by the saturation state of dietary fat. A defect in processing of membranes of lysosomes, melanosomes, and dense bodies may be involved in the syndrome.

Immunology

CHARACTERIZATION OF BIOLOGICAL AND BIOCHEMICAL PROPERTIES OF MELANOSOMAL PROTEIN IN HUMAN MALIGNANT MELANOMA BY DEVELOPMENT OF MONOCLONAL ANTIBODY, MoAb HMSA-1:

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A mouse monoclonal antibody (MoAb) of HMSA-1 (Human Melanosome Associated Antigen) was developed against the melanosome (MS) fraction of human malignant melanoma (MM). It characteristically identifies neoplastic melanocytes (MC), and may differentiate even between benign and malignant conditions. It does not react with normal MC nor does it react with any embryonic tissues (Akutsu Y & Jimbow K, Cancer Res, in press). This study characterizes the biological and biochemical properties of HMSA-1 and its synthetic pathway by enzyme linked immunosorbent assay and immunoelectron microscopy. We found (a) that HMSA-1 is highly present in the fractions of MS and endoplasmic reticulum (ER), (b) that it is localized in possibly Stage I MS, and smooth ER, (c) that it is not tyrosinase because of lack of enzyme activity and different MW, and (d) that HMSA-1 is highly purified by successive steps of ionic exchange column, immunosorbent column of MoAb HMSA-1 and HPLC (protein pak, DEAE 5PW). It is assumed that HMSA-1 is a structural matrix protein which is produced by a synthetic pathway different from tyrosinase and which is a new class of differentiation antigen for neoplastic MC.

THYMOSINS: MODULATORS OF IMMUNE AND NEUROENDOCRINE CIRCUITS. Allan L. Goldstein, P. Naylor and H. Hall, Department of Biochemistry, The George Washington University School of Medicine, Washington, D.C. 20037. The central role of the thymus in regulating the immune system is now well established. It acts as the master gland of immunity by producing hormonal-like peptides such as the thymosins and by the seeding of T-cells. The thymus accomplishes control over immunity by influencing the functioning of T-cells and by the feedback loops to the brain. Most recently, it has been found that a number of thymosin peptides can stimulate the release of neuropeptides such as ACTH, LRF, LH and β-endorphin, thus establishing for the first time a direct link between the endocrine thymus and the neuroendocrine system. Our data support the hypothesis that there are bidirectional circuits between the central nervous system (CNS) and the immune system. Soluble products that appear to transmit information from the immune compartment to the CNS include thymosins, lymphokines, cytokines and a number of other biological response modifiers (BRMs). BRMs hold much promise clinically as potential therapeutic agents in the treatment of diseases ranging from cancer to AIDS. Recent studies have shown that neuropeptides such as opoid peptides, ACTH and TSH are also produced by lymphocytes and may function in immunomodulatory neuroendocrine circuits. It is proposed that the term IMMUNOTRANSMITTERS be used to describe those substances that can be produced by cells within the immune compartment, but which have biological functions in the CNS other than those originally ascribed to them.

MACROPHAGE ACTIVATION IN NUDE MICE BY HUMAN MALIGNANT MELANOCYTES. J.F. Doré and A. Benomar, INSERM U. 218, Centre Léon Bérard, Lyon, France

Interactions of nude mouse macrophages with human melanoma cell lines (HMCL) characterized by their varying ability to grow in nude mice were investigated. HMCL were found to activate nude mice macrophages which became tumoricidal for EL4 target cells. However, the kinetics of this activation depended on the tumorigenicity of the cell line used. Inoculation of a poorly tumorigenic cell line (PTCL) yielded a higher and more lasting activation than inoculation of a highly tumorigenic cell line (HTCL). Increasing the number of melanoma cells resulted in a parallel increase in the cytotoxicity of macrophages when activated by PTCL and in a decrease when activated by HTCL. HMCL were found to be equally susceptible in vitro to cytostatic and tumoricidal activities of nude mice macrophages activated in vivo with Brucella abortus B19R. But in cross-experiments using PTCL-activated macrophages as effectors HTCL cells were found to be less sensitive than PTCL cells. Peritoneal cells rich in macrophages activated in vivo eitner by Brucella abortus or by HMCL prevented the growth of HTCL in nude mice in a Winn assay. These data demonstrate that HMCL could activate nude mice macrophages in vivo and suggest that the ability of human tumors to grow in nude mice could be related to their capacity to activate host's macrophages and to their susceptibility to the tumoricidal activity of activated macrophages.

MURINE MELANOMA SPECIFIC TUMOR REJECTION ACTIVITY ELICITED BY A PURIFIED MELANOMA ASSOCIATED ANTIGEN. Vincent J. Hearing, Douglas M. Gersten, Paul Montague, Wilfred D. Vieira, Giorgio Galetto, and Lloyd W. Law, Lab. of Cell Biology, NIH, Bethesda, MD, 20892 U.S.A, and Dept. of Pathology, Georgetown Univ. Med. Center, Wash., D.C., 20005 U.S.A.

We have characterized a melanoma specific, 65,000 dalton glycoprotein antigen (B700), which has a pI of 4.5, and extensive sequence homology to albumins (PNAS 78:5109; BBRC 121:196). The synthesis and expression of B700 is restricted to all murine melanomas tested, and the capacity of this purified antigen to function as a tumor specific transplantation antigen (TSTA) has been demonstrated in this study. Mice were immunized 3 times with varying doses of B700, and subsequently challenged either SC or IV. We found that immunized mice were able to significantly inhibit the growth of primary B16 tumors, and of metastases. Immunization with this TSTA also protected against challenge with JB/RH and K1735 murine melanomas, but was specific to melanomas in that two other tumors, the RBL-5 leukemia and the MCA-105 sarcoma were not affected. B700 has been shown to be unrelated to other known murine tumor antigens, or to murine leukemia virus antigens. B700 immunized mice produced specific antibodies, but our immunofluorescence studies revealed that not all B16 melanoma cells expressed similar levels of B700 on their cell surface. Whether the heterogeneity in the expression of this antigen is related to problems in the immunologic management of tumor growth by the host has not yet been determined.

IMMUNOGENEIC B16 MELANOMA ANTIGENS. Dean Johnston and Jean-Claude Bystryn, Kaplan Cancer Center and NYU School of Medicine, New York, N.Y.

Murine B16 melanoma, a tumor widely used to study immunity to melanoma, expresses antigens that can induce both humoral and tumor protective immunity. However, the identify of these important antigens is not known. To answer this question, immune sera obtained from mice immunized to a B16 melanoma vaccine which induces tumor protective immunity were used to immunoprecipitate detergent lysates of radioiodinated B16 melanoma cells. Immunogenic antigens were identified by SDS-PAGE and autoradiography of immunoprecipitates. Immune, but not control, sera precipitated three surface antigens with molecular weights of approximately 90, 200 and 250+ kd. The three antigens were expressed by several variants of B16 melanoma but not by unrelated syngeneic or xenogeneic cancers or by normal murine tissues. There was an inverse relation between the titer of melanoma antibodies induced by immunization to these antigens and subsequent tumor growth.

Thus, we have identified some of the B16 melanoma antigens which induce anti-tumor immune responses in syngeneic mice. The correlation between the magnitude of immune responses induced by these antigens and tumor growth suggests these antigens are also of importance in tumor protective immunity.

EXPRESSION AND MODULATION OF A B16 MELANOMA ASSOCIATED ANTIGEN. Stanley P. L. Leong[1], Elieser Gorelik[2], Jack A. Roth[1], and Vincent J. Hearing[3]. [1]Surgery Branch, [2]Biological Therapeutics Branch, and [3]Laboratory of Cell Biology, NCI, NIH, Bethesda, MD 20892

Two monoclonal antibodies (MoAb), an IgG_{2b} and an IgM, have been produced against a melanoma associated surface antigen (MAA). Data from immunofluorescence studies showed that the MAA was expressed on the JB/RH and on 6 sublines of the B16 murine melanoma. This MAA was not detectable on 3 other murine melanomas, 11 murine nonmelanoma tumors, 9 syngeneic normal murine tissues, or 13 human melanomas examined. Expression of this MAA was compared to that of $H2^K$ and $H2^D$, and to synthesis of pigment. Cultured cells were harvested and reacted with MoAb specific for MAA, $H2^K$, or $H2^D$, and binding analyzed by fluorescence flow cytometry. No correlation of the expression of MAA with H2 on the cell surface, or with the production of tyrosinase or melanin, was found on 6 B16 sublines. MAA expresssion was maximized as the cells reached confluence (~98% positive), but decreased afterwards (~20% positive 12 d later). Dual parameter staining showed that the nonantigenic cells were in G_0/G_1. Levels of surface $H2^K$, and tyrosinase activity, declined similarly (~5 fold), but $H2^D$ levels were relatively constant. EM histochemical analysis demonstrated that once the MoAb were bound to the surface MAA, they were internalized rapidly. In light of the correlation of the cell cycle with expression of MAA, its specificity, and its rapid internalization, this MAA should be an excellent model for the study of tumor antigens, their regulation by environmental conditions, and the effective immunomodulation of tumor growth by immunocytotoxic agents.

COEXPRESSION OF HLA-DR AND MELANOMA-ASSOCIATED ANTIGENS DURING THE CELL CYCLE IN HUMAN MELANOMA CELL LINES. Koichiro Kameyama, Takeshi Tone, Shin-ichiro Takezaki, Tamotsu Kanzaki, and Kohzoh Imai. Dept. of Dermatology and Internal Medicine, Kitasato Univ. and Sapporo Med. College, Sagamihara and Sapporo, JAPAN

The expression of HLA-DR and melanoma-associated antigens (MAA) was investigated in six human melanoma cell lines. Cells were cultured with or without gamma interferon for three days. Monoclonal antibodies used for these studies were, 96.5, 225.28S and L243, which recognize p97, high MW-MAA and HLA-DR, respectively. Cells were initially stained with 96.5 or 225.28S, and then stained with L243. These dual stained cells were analyzed with FACS. All cell lines expressed these antigens to a various degree, with or without treatment with gamma-interferon. There was a strong correlation between the expression of these two antigens, so that HLA-DR positive melanoma cells tended to express MAA, and vice-versa. Cells were stained first with 96.5 or L243, and then stained for DNA with propidium iodide. Flow cytometric analysis showed that gamma interferon increased the density of these antigens throughout the cell cycle, and that the density of these antigens on the cell surface remained relatively constant throughout the cell cycle, and both antigens were maximally detected during the G_2-M phase, whether these cells were treated with gamma-interferon or not. Therefore our results clearly suggest that expression of surface MAA and HLA-DR are closely linked to the cell cycle.

POTENTIATION OF GROWTH SUPPRESSION AND INDUCTION OF DIFFERENTIATION IN HUMAN MELANOMA CELLS BY THE COMBINATION OF FIBROBLAST AND IMMUNE INTERFERON.

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In addition to its antiviral activity, recent studies indicate that specific interferons are potent modulators of differentiation in several biological systems. In the human melanoma cell line HO-1, inhibition of growth by recombinant human fibroblast interferon (IFN-β) is associated with an increase in melanin synthesis and alterations in the expression of class 1 HLA antigens and tumor associated antigens (TAA). In the present study we have evaluated the effect of recombinant human leucocyte interferon (IFN-α), immune interferon (IFN-γ) and IFN-β, alone and in combination, on growth, differentiation and antigen expression in HO-1 cells. IFN-β was the most active interferon preparation in inhibiting growth and inducing melanin synthesis. When IFN-β was combined with IFN-γ, growth suppression and differentiation were potentiated, whereas IFN-α plus IFN-γ was less effective in inducing these changes in human melanoma cells. Changes in antigen expression were also observed, some of which may be related to the state of differentiation of HO-1 cells. This study provides further support for the hypothesis that suppression of tumor cell growth by interferon may in some cases be related to induction of terminal differentiation.

DETECTION OF MELANOMA-ASSOCIATED ANTIGEN p97 ON HUMAN MELANOMA CELL LINES.

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p97, a 97,000 molecular weight cell surface glycoprotein, is one of the most representative melanoma-associated antigens(MAAs) expressed on most human melanomas. The purpose of the present study is to examine the expression of p97 antigen on human melanoma cell lines using mixed passive hemagglutination(MPHA).

MATERIALS AND METHODS: The expression of p97 antigen was investigated on five human melanoma cell lines M-AS, Seki, Endo, HMV-I and HMV-II. The monoclonal antibodies to $p97^a$ and $p97^b$ were purchased from Hybritec Inc.,USA. A modified MPHA method(Saida,T. et al.: Jpn.J.Dermat., in press) was used for the detection of p97 antigen.

RESULTS: The expression of p97 antigen was demonstrated on M-AS, Seki, Endo and HMV-II but no on HMV-I. These results were confirmed by immunoelectron microscopic observations. HMV-I and HMV-II are subclones separated from a common original cell line and differ in cell type and melanin production. In addition, we found that OKB2, a B cell marker, was expressed on HMV-I but not on HMV-II.

CONCLUSION: Clonal variation in the expression of MAA on melanoma cell lines as shown in the present study may offer serious problem when monoclonal antibody to MAA is used for immunodiagnosis or immunotherapy.

DETECTION OF ANTIGEN IN THE SKINS OF COAT COLOR MUTANT MICE BY MONOCLONAL ANTIBODY AGAINST MELANOSOME

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Among coat color genes in mice, some loci are known to control melanosome formation. In order to investigate genetic control on melanosome-associated proteins, we prepared monoclonal antibodies against mouse melanosomes. Melanosomes were isolated through cell fractionation of B16 mouse melanoma. BALB/c mice were immunized with SDS-solubilized melanosome fraction. The spleen cells were subsequently fused with P6 mouse myeloma cells, the resulting hybridomas were cloned, and then the secreted IgG was screened by ELYSA method using SDS-solubilized melanosome fraction. One such monoclonal antibody, M10, gave staining of melanosomes with immuno-electron-microscopy. It recognized single protein band, with an estimated M.W. 61,000, on immunoblots of gel-fractionated melanosome fraction. The reactivities of M10 to skin homogenates from several mutant mice were detected by ELISA. Five congenic genotypes, non-agouti (a/a), brown (b/b), albino (c/c), dilute (d/d) and pink-eyed dilution (p/p) were examined. Among these, b/b and p/p exhibited significantly lower reactivities than a/a. Our results seem to suggest that the ultrastructural abnormalities of melanosomes in these mutants result from the abnormalities of the antigen.

Photobiology

CHANGES IN THE PIGMENT LEVEL AND ASSOCIATED CHANGES IN MED IN PERSONS RECEIVING UVB TREATMENT FOR PSORIASIS.

A.Baqer, I.Al Awadi, N.Kollias Dermatology Department, Al Sabah Hospital, Physics Department, Kuwait University, Kuwait.

Each individual involved in this study was tested the first day of each week and their MED was assessed 24 hours later. He then received treatment at the MED level for the remainder of the week. At the beginning of the second week he was phototested once again to determine his new MED. At each instance that the MED was determined the pigment level was also assessed. The subjects were ten psoriatic individuals who had been selected for UVB treatment.

We find that the MED level increases for the first two to three weeks, as expected, and then it remains constant for the following five weeks. In several individuals we found that the MED decreased after the fourth week of tretment. We also find that the pigment level increases in the first few weeks and it arrives at a plateau. The correlation between the detailed variation in the pigment level and the variation of the MED will be discussed. We have found that the initial MED before the treatment is commenced is very weakly correlated with the native melanin level, we are thus unable to predict the MED based on the native melanin level.

We thus find that frequent phototesting is necessary for individuals that are genetically capable of pigmenting, if the treatment is to remain effective and the individual is to not be overdosed.

MELANIN CONTENT AND THE SENSITIVITY OF MELANOMA CELLS TO HYPERTHERMIA AND RADIATION

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Previous experiments have indicated that pigmented Bomirski hamster melanoma (BHM) cells are more radioresistant than non-pigmented ones (B. Iwasiow, M. Kapiszewska, IV Symp. Polish Soc. Med. Phys. 1975, p. 16; M. Kapiszewska, B. Iwasiow, S. Lukiewicz, IV Meeting Polish Soc. Rad. Res. 1976, p. 8; K. Cieszka, R. Gurbiel, S. Pajak, VII Intern. Congr. Rad. Res. 1983, DI-05). The present work aims at testing whether hyperthermia can modify the radiosensitivity of pigmented BHM cells. Pigmented and non-pigmented BHM cells growing in primary cell cultures were heated at 45,5°C for 5 minutes and then irradiated with different doses of X-rays (220 kV). Immediately after irradiation the cells were implanted intradermally into the skin of a golden hamster. The kinetics of tumor growth was followed over a period of 0.5 - 3 months. The calibration curves showing the relation between tumor size and the number of implanted cells were used to estimate the number of cells capable of dividing after implantation. Survival curves show that the D_0 values for BHM cells irradiated after hyperthermia are lower than D_0 for cells irradiated without heating.The combined action of hyperthermia and X-rays in the case of pigmented BHM cells reduces survival by 12 times but only by 2,4 for non-pigmented ones.The conclusion is that hyperthermia is 5 times more effective as a radiosensitizing agent versus pigmented BHM cells in comparison with their amelanotic counterparts.

EFFECT OF IN VITRO EXPOSURE TO X-RAYS AND HYPOTHERMIA OF S91 CLOUDMAN MELANOMA CELLS WITH DIFFERENT MELANIN CONTENT

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Previous experiments have shown that pigmented melanoma cells irradiated at 4°C are more sensitive to radiation than those irradiated at room temperature (Hill Z., Hill G.J., Miller C., Kwong F., Purdy J., Rad. Research 90, 259, 1979; Cieszka K., Kapiszewska M., Gurbiel R., Hyrc K. Annual Meeting Europ. Soc. Radiation Biol., Prague, 1985, p. 41, 42).
The question arises as to whether this effect has any relation to the intracellular content of melanin. To solve this problem the radiosensitivity of S91 Cloudman melanoma cells with a different melanin content was checked after X-ray irradiation at 20°C and 4°C. The amount of melanin was modified by the in vitro treatment of cells with 2.10^{-7} M MSH for 48 hr. The survival of cells after irradiation was estimated by the method of cell cloning.
It was found that (1) an increase in pigmentation brings about a decrease in radiosensitivity of melanoma cells independently of the temperature of irradiation, (2) only cells with a high melanin content are sensitized to X-rays by hypothermia combined with irradiation.
It is concluded that some of the factors which determine the amount of melanin in the cell (e.g. tyrosinase activity) may also affect its response to radiation and hypothermia, perhaps by modifying the concentration of dissolved oxygen.

EUMELANIN ENHANCES DNA STRAND BREAKS CAUSED BY IONIZING RADIATION IN B16CL4 MELANOMA CELLS.

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Skin cancer may be caused by active oxygen--species and free-radicals formed in the cell nucleus by sunlight. Melanins may protect against such carcinogenic DNA damage by screening and by radical scavening. They have also been shown to produce DNA damaging species on illumination. Therefore, their form, concentration and packaging is critical in determining whether they will be beneficial or deleterious. In these experiments, dialyzed eumelanin synthesized by autooxidation of DOPA was incubated in growth medium with B16CL4 mouse melanoma cells for varying time periods and at varying concentrations. Under these conditions, there was a concentration and time dependent increase in DNA strand breaks induced in the cellular DNA by ionizing radiation as measured by alkaline elution - although the melanin alone had no effect on strand break induction. High concentrations of melanin in the cold irradiation buffer had no effect on the induction of strand breaks, suggesting that the melanin must penetrate into the cell, and probably into the nucleus, to be effective. These findings suggest that eumelanin absorbs free-radicals produced by ionization of water and emits sufficient active oxygen species to overwhelm the cell's capacity to detoxify them with superoxide dismutase and catalase. Eumelanin may be a two edged sword, either enhancing or reducing free-radicals in the nucleus.

HOW RELIABLE IS SKIN TYPING ACCORDING TO BURNING-TANNING HISTORIES ?

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Melanoma risk is closely associated with skin type, which is usually determined by the self-reported tendency to burn versus tan following ultraviolet exposure (skin types I-IV after Fitzpatrick). Burning-tanning histories, however, may be very subjective parameters. We studied the association between burning tendency after one hour sun exposure in early summer and tanning ability after gradual and repeated sun exposure (scoring indices 0 = none, 1+ = mild, 2+ = moderate, 3+ = severe). 389 young adult whites of 18-30 years participated in the study. Only 168 cases (43.2%) were classifiable according to the Fitzpatrick rules. Skin type I (always burn - never tan) was not recorded at all. Subjects tended to overrecord "no burning" (171 cases, 44.0%) and to underrecord "no tanning" (2 cases, 0.5%). Several cases with "no burning" skin complexion nevertheless affirmed that they had suffered severe sunburn regularly during their life (14.0% three times or more). Surprisingly, in the "no burning" group 82.2% admitted that they used sunscreens to protect themselves from sunburning (47.9% sometimes and 34.3% even regularly or often). It is concluded that burning-tanning histories merely reflect the present-day vogue of sun-worship and are not useful in delineating risk categories of cutaneous melanoma.

THE EFFECTS OF HYPERTHERMIA AND X-RAYS ON THE ELECTRIC PROPERTIES OF HAMSTER MELANOMA CELL SURFACE

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It has been reported that the electrophoretic mobility of pigmented Bomirski hamster melanoma (BHM) cells is reduced by ionising radiation but not by hyperthermia. In contrast, both X-rays and hyperthermia modify the electrophoretic structure of the BHM cell population. The present work aims at testing the effects of the combined action of hyperthermia and X-rays on the profile of the electrophoretic mobility (EPM). In vitro growing BHM cells were heated at 45,5°C (0 - 15 min.), irradiated with X-rays, and implanted intradermally into the skin of golden hamsters. The EPM was measured immediately after irradiating BHM cells, and on their progeny derived from the in situ growing tumors. It was found that irradiation of BHM cells with 2 Gy of X-rays does not result in any significant change of the EPM unless it is preceded by hyperthermia. The minimal dose which causes a reduction in the EPM of the progeny of irradiated cells is much lower for cells which have been heated before irradiation than for those which have only been irradiated. The electrophoretic structure of the BHM cell population developing in situ from heated and irradiated cells does not differ significantly from that of cells growing from heated cells. We conclude that X-rays reduce the EPM of heated BHM cells more effectively than that of non-heated ones but has no effect on the electrophoretic structure of a heated BHM cell population.

THE ABSORPTION CHARACTERISTICS OF HUMAN MELANIN IN THE VISIBLE. N.Kollias, A.Baqer. Physics Department, Kuwait University and Dermatology Department, Al Sabah Hospital, Kuwait.

We have shown that the absorbance of human melanin in vivo, in the range 620-720nm can be used to assess the melanin concentration in skin, as it is perceived by the eye (We call this Type I absorbance). This means that a unique parameter can be determined for each skin sample that is directly related to the melanin concentration.

In the wavelength range 620-720nm we found a linear depencdence of the absorbance on wavelength (type I). In the wavelength range 400-500nm we find an exponential dependence of the absorbance on the frequency of light, (type II absorbance). This behaviour is explicit in all normal individuals except for the extremely light and extremely dark, in these two cases we are limited by instrumental parameters. The form of melanin that is responsible for this part of the absorbance is the same for all individuals studied, it simply appears in different amounts in different people and this variation is NOT perceptible by eye!

We have found NO correlation between the two types of absorbance, neither when we consider a number of individuals (75) assessed at the same anatomical site nor when we consider various anatomical sites (18) of the same individual. Type II absorbance appears to be dependent on concentration and the available Oxygen to the epidermal melanin.

IMMEDIATE PIGMENT DARKENING (IPD) : SPECTROSCOPY AND DEPENDENCE ON ATMOSPHERIC GASES. N.Kollias, A.Baqer. Physics Department, Kuwait University and Dermatology Department, Al Sabah Hospital, Kuwait.

UVA induced IPD reaction has been studied with doses between five and fifty Joules / square centimeter. The spectroscopic characteristics of immediate pigment darkening are determined by comparing the irradiated skin site with an adjacent non-irradiated site. We make certain that the pigmentation is uniform on both sites before the irradiation.

We find that the absorbance of the skin increases over the entire visible range (400-720nm). The curve that we obtain resembles the absorbance of melanin in the 620-720nm range and appears as an increase of the concentration of that type of melanin. In the range 400-500nm we find a negative change in absorbance. This we interpret as a depletion of the absorber, the type of melanin that is responsible for the shorter wavelength absorption. It appears that the long wavelength absorption is increased at the expense of the short wavelength absorption. We find that UVB induced delayed pigment (DP) appears as an increase in both components i.e. there is neomelanogenesis.

We also find that circulating pure oxygen or pure nitrogen in the exterior of the skin prior to irradiation has a significant effect on the IPD reaction, as compared to no circulation. The area exposed to oxygen has a measurtably stronger IPD reaction while the nitrogen area has a suppressed reaction.

THE EFFECT OF PHOTOTOXIC DRUGS ON THE EPIDERMAL MELANOCYTES IN PHOTOCHEMOTHERAPY

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The combining of subsequent ultraviolet light exposure with the administration of phototoxic drug is now accepted as an important treatment for vitiligo and psoriasis, etc. Currently the most commoly used phototoxic agents are 8-methoxy psoralen (8-MOP) and 4,5',8-trimethyl psoralen (TMP).

The purpose of this study was to compare the effect of these two agents on the number and size of melanocytes in C57 BL black mice. Intraperitoneal injecting of the agents followed by UV-A irradiation were done twice weekly for 8 weeks in small doses and 5 weeks in large doses. Split DOPA stain was made for measuring the number and size of melanocyte weekly.

In descending order, the groups in which the greatest numbers appeared were TMP-large dose, 8-MOP-large dose, TMP-small dose, and 8-MOP-small dose. Also in descending order, the groups in which the greatest number of melanocytes underwent an increase in size were TMP-large dose, TMP-small dose/8-MOP-large dose, and 8-MOP small dose.

To summarize, TMP is superior to 8-MOP in increasing the number and stimulating the morphologic development of melanocytes.

PHOTOBIOLOGY OF MELANIN PIGMENTATION IN HUMANS OF SKIN TYPES I - VI. MA Pathak and TB Fitzpatrick, Dept Dermatology, Harvard Med School, Boston, MA.

The ultraviolet dose-response relationship for 24-hour delayed erythema and 5- to 7-day pigmentation response (melanogenesis) in over 50 individuals of Skin Types I - VI has been examined. The minimal erythema dose (MED) and minimal melanogenic dose (MMD) values for the UVB spectrum (290 - 320 nm) and UVA spectrum (320 - 400 nm) were as follows:

Skin Type	UVB (290-320 nm) MED (mJ/cm²)	UVB (290-320 nm) MMD (mJ/cm²)	UVA (320-400 nm) MED (J/cm²)	UVA (320-400 nm) MMD (J/cm²)
I	20-30	—	20-35	—
II	25-35	15-25	30-45	15-20
III	30-50	17-25	40-55	20-30
IV	45-60	20-30	50-80	20-40
V	60-80	30-35	70-100	30-55
VI	80-200	40-100	>100	30-45

The data indicate: a) MED values of the UVB spectrum for Skin Types I - IV are about 700 to 1,000 times less than UVA MED values; b) Skin Type I burn but do not tan; c) MMD for Skin Type II is about the same as MED; d) MMD for Skin Types III, IV, V, and VI is significantly less than their MED, both for UVB and UVA spectra; and e) UVA stimulates proliferation of melanoctyes and increased production of melanosomes with little or no evidence of erythema and epidermal cell hyperplasia. Data indicate melanogenesis can be stimulated by suberythemogenic doses of UVB and UVA in Skin Types II - VI. Sun protection factor values for melanin (constitutive pigmentation) in Skin Types II - VI range from 1.5 to 5.0 (supported by NIH grant 2-R01-CA-05003-28).

SUN EXPOSURE HABITS IN PATIENTS WITH GALLSTONES. PRELIMINARY RESULTS OF A CASE CONTROL STUDY.

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Cholelithiasis is known to be one of the most frequently occuring diseases. However, the pathogenesis of gallstone formation is still not fully understood. Particularly the factors playing a role in the initiation of bile concrement formation are unclear. Our new theory on the gallstones pathogenesis suggests that the activation of melanogenesis in pigment cells may result in the increased concentration of melanin precursors in bile and their subsequent polymerization i.e. the formation of the nucleus of the future bile concrement. The activation of melanogenesis by sun light may therefore be considered as a possible risk factor for gallstone formation. We studied the solar exposure habits of a group of patients with gallstones and compared the data with those obtained from the group of age- and sex-matched controls. People with positive attitude to sunbathing (1), having skin type I or II (2) spending sometimes vacations in sunny areas (3) and using no protective sunscreens (4) were considered as being at risk, if at least 3 of the mentioned conditiones were met. People considered as running no-risk met only one of the four mentioned conditions. Our preliminary results show that people at risk have increased incidence of gallstone disease (odds ratio 2.85).

INDIRECT EFFECT OF UVB-IRRADIATION ON THE MELANOCYTE SYSTEM IN COVERED SKIN

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Twelve healthy volonteers (skin type II-IV) received total body UVB-irradiation 8 times during 17 days. The UV dose was the same for all individuals and was increased to give a slight erythema for skin type II at all exposures. During the irradiation a 225 cm^2 large area of the left buttock was protected with a non transmitting material. Biopsies were taken before and 3 weeks after the last UVB exposure from covered and irradiated buttock skin. The melanocyte population density was estimated in DOPA incubated split skin preparations by a random-dot procedure. (1)
The melanocyte population increased in both irradiated (2-fold) and protected (1.5-fold) skin. The increase in the protected skin is probably due to an UVB induced release of a systemic mitogenic factor as earlier observed in mice. (2)
The existence of such a mechanism also in humans might be a link between UV exposure and melanoma development in covered skin.

Ref.: 1. Rosdahl I, Rorsman H, Pigment Cell 1985, Biological, Molecular and Clinical Aspects of Pigmentation, p. 555-561.
2. Rosdahl I, J. Invest, Dermatol. 73:306-309, 1979.

THE OZONE DEPLETION MELANOMA CONNECTION.
LET'S HELP THE WORLD METEROLOGICAL ASSOCIATION MORE.

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N.A.S.A. has been in the forefront of ongoing studies about man-caused depletion of stratospheric ozone, an increasing problem which affects the health of people the world over. Many meetings have been held in order to study this problem, and to take measures to deal with it effectively. Inasmuch as it is ozone alone which absorbs UVR in the 240-320 nm wavelengths, its continued depletion poses an increasingly significant risk factor for the development of malignant melanomas in humans.

In addition to controlling the pollution of our stratosphere by international regulation of those substances known to deplete atmospheric ozone, the author suggests that the IPCS could play an increased role in joining future talks between men of science and men of government by stressing the significance of the spiralling escalation of melanoma in recent years, probably a direct result of inadequate control of such pollution.

He also suggests asking the scientific community if a cost-effective way of manufacturing ozone and delivering it into the stratosphere might be developed, and then to do so, if feasible. If not, to try.

It is at least possible that an international concern about a little black spot in the skin which can be deadly might represent a gigantic step forward in our ongoing quest for better understanding between the nations of the World.

INCREASE OF TYROSINASE BY UV-B IRRADIATION IN HUMAN CULTURED MELANOCYTES FROM SUCTION BLISTERS

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The mechanism of delayed-type skin darkening by UV irradiation has not been elucidated yet. There may be two hypotheses about the mechanism of UV-induced melanogenesis. One is that UV stimulates melanocytes directly to increase the synthesis of melanin and the other is that cells around the melanocytes in the skin may release melanotropic factor(s) by UV stimulation and the factor(s) induce(s) the melanogenesis in the melanocytes.
The direct effect of UV irradiation on the melanogenesis was examined by using the culture of melanocytes obtained from the roofs of suction blisters produced on the skin of adult oriental volunteers. Since the epidermal roofs of blisters did not contain any fibroblasts, and since keratinocytes did not attach to the culture dishes in the presence of phorbol 12-myristates, many melanocytes were obtained without contamination of other cells and direct effect of UV irradiation on the melanocytes could be observed. Irradiation of UV-B (26 mjoule/cm^2 in all) using FL20SE lamp increased tyrosinase in the melanocytes, which was shown by the increasing intensity of the immunofluorescent staining using anti-tyrosinasr monoclonal antibody. This indicates that UV-B directly stimulates the synthesis of tyrosinase and increases the melanogenesis in the melanocytes in the human skin.

SENSITIVITY OF MOUSE SKH:HR-2 TO ULTRAVIOLET LIGHT: PIGMENTATION KINETICS. Raphael Warren, Paul A. Gardner, and Jody C. Reed, Beauty Care Division, The Procter and Gamble Company, Cincinnati, Ohio, U.S.A.

The hairless mouse, Skh:HR-2 was exposed to ultraviolet light over a two-week period at UV-doses known to induce pigmentation ($100mJ/cm^2$ UVR and $300mJ/cm^2$ UVR). The kinetics of pigmentation during this two-week period were monitored using three independent methods:
a) spectrophotometric analyses of skin darkening;
b) biochemical analysis of skin melanin; and
c) histological analysis of DOPA-stained epidermal cells.

Within 2-24 hours of the first UV-exposure the skin became lighter. This was associated with both a reduction in skin melanin content and a reduction in the number of DOPA-stained epidermal cells. The degree of skin lightening, and the reduction in melanin and the number of DOPA-stained cells were related to the UV-dose. Over the next two weeks, skin pigmentation increased. Perturbations of each parameter were directly related to both the duration of UV-exposure and the UV-dose. Since the histological DOPA assay may be interpreted as a microtyrosinase assay, the results suggest that preceding the melanin inducing effect of UV-irradiation, melanocytes may be sensitized to UV-irradiation resulting in their immediate inactivation.

STUDIES ON THE MECHANISM OF ENHANCED PIGMENTATION IN TRANSFORMED MELANOCYTES TREATED WITH 8-METHOXYPSORALEN (8-MOP) AND UVA LIGHT. Edward J. Yurkow and Jeffrey D. Laskin. UMDNJ-Rutgers Medical School, Piscataway, N.J. 08854

Psoralen (8-MOP), in combination with UVA light (PUVA), is a potent inducer of skin tanning. To study the mechanism of PUVA induced pigmentation, we used B16/C3 melanoma cells grown in vitro. 8-MOP (1 μM) and UVA ($0.69\ J/cm^2$) were found to increase melanin production by the cells 3-4 days following treatment. Enhanced melanogenesis was associated with a 4-fold increase in tyrosinase activity in the cells. This increase was determined to be due to altered kinetics of enzyme turnover and isozyme biosynthesis. Using a highly specific antibody produced in our laboratory, tyrosinase was immunoprecipitated from cells pulse-labeled with ^{35}S-methionine. PUVA increased the rate of total enzyme synthesis and, at the same time, decreased enzyme degradation. Untreated cells produced at least four distinct tyrosinase isozymes ranging in molecular weight from 50,000 to 70,000 daltons. PUVA increased the levels of these isozymes in the cells and induced the production of several additional isozymes with molecular weights ranging from 69,000 to 90,000 daltons. These data demonstrate that alterations in tyrosinase turnover and isozyme patterns are responsible for enhanced pigmentation induced by PUVA in B16/C3 melanoma cells. Supported by NIH grant ES 03647.